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SCHOOL OF GRADUATE STUDIES

**Evaluation of Essential Oils of Some Local Plants
for their Repellency against *Anopheles arabiensis*
and *Aedes aegypti***

**A Thesis Submitted to the School of Graduate Studies
in Partial Fulfillment for the Degree of Master of
Science in Biology**

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ABSTRACT

Essential oils of six aromatic plants: *Ocimum suave*, *O. lamiifolium*, *Eucalyptus citriodora*, *E. globulus*, *Lippia adoensis* and *Schinus molle* were evaluated for their repellent activity against *Anopheles arabiensis* and *Aedes aegypti*. The experiments were conducted in the laboratory established at Aklilu Lemma Institute of Pathobiology using human volunteers. Each volunteer used his left arm for control and right arm for treatment and area from wrist to fingers was covered by surgical glove. First, the control arm was exposed to 30 nuliparous mosquitoes starved for 3-6 days inside a screened cage and the landing/biting mosquitoes were counted in 30 seconds. And immediately thereafter, the treated arm was exposed in the cage for the same 30 seconds and the landing/biting mosquitoes were counted and percentage of protection was computed.

At 20% concentration, percentages of protections of the test oils were compared with the standard synthetic DEET. In case of *An. arabiensis*, DEET provided 83-100% protection for 6 hours, *O. suave* 83-100% for 4 hours, *L. adoensis* 89-100% for 2 hours, and *E. citriodora*, *E. globulus*, *S. molle*, but *O. lamiifolium* did not provide any protection even for an hour. In case of *Ae. aegypti*, DEET provided 84-100% protection for 6 hours, *O. suave* 85-100% for 5 hours, *L. adoensis* for an hour, and the rest four oils did not provide protection even for an hour. At 10% concentration all the test oils did not provide protection for an hour against both *An. arabiensis* and *Ae. aegypti* except *O. suave* which provided 88-100% protection for 3 hours against *Ae. aegypti*. In the study of repellency of blend effects of the six oils against *An. arabiensis*, *O. suave* + *L. adoensis*, *O. suave* + *E. citriodora*, *O. suave* + *E. globulus*, *O. suave* + *S. molle* and *O. suave* + *O. lamiifolium* provided over 84% protection for 3 hours, and the other blends without *O. suave* provided protection for less than and nearly an hour at 20% concentration, and blend effects of the five oils with *O. suave* provided protection for an hour, where as the other blends other than *O. suave* provided less than an hour protection at 10% concentration.

All six essential oils and blends exhibited repellent properties in various degrees to adult female mosquitoes of *An. arabiensis* and *Ae. aegypti*. But, of the six oils *L. adoensis* against *An. arabiensis*, *O. suave* against *An. arabiensis* and *Ae. aegypti* and 1:1 combinations of *O. suave* with five oils against *An arabiensis* showed remarkable repellency. However, field investigation is required to ascertain the relative potential of repellency of *L. adoensis*, *O. suave* and mixtures of *O. suave* and the other oils against *An. arabiensis*, *Ae. aegypti* and other vector mosquitoes.

1 INTRODUCTION

1.1 Global malaria situation

Malaria remains one of the major determinants of ill health in many countries of Africa, Asia and Latin America. In Africa south of Sahara, malaria is the leading health problem, with almost the entire population being at risk. More than 74% of the population lives in highly endemic areas while about 18% lives in epidemic prone areas and only 7% lives in low or malaria free areas. The annual clinical cases are in the magnitude of 350-500 million with one to three million deaths a year, most of them are among children under the age of five (MOH, 2002; WHO, 2002; Giha *et al.*, 2005; Morlais *et al.*, 2005). Pregnant women are also more prone to severe attacks of malaria (Prasad *et al.*, 1990).

In spite of the present control measures (use of insecticides and ITNs for vector control and use of antimalarial drugs for the control of malaria parasites), the situation is worsening (Negash *et al.*, 2005) with the evolution of resistance to cheap and easily available drugs and insecticides, changes in the environmental conditions leading to increasing epidemics, civil unrest coupled with population movements and economic development programmes in risk areas such as wetlands, desert fringes, and high lands. Indeed malaria has spread into areas, which previously had low or no transmission (MOH, 2002). For instance, during the 1970s and 1980s, the resurgence of imported malaria in some of the developed countries, such as the U.S.A., Europe and the U.K. was reported (Gilles and Warrel, 1993).

The epidemic of malaria is increasing due to widespread of resistant malaria parasites to antimalarial drugs and the vectors to insecticides making malaria control complex (WHO, 1996; 2001). Chloroquine-resistant falciparum malaria first occurred in South American and in Indo-Chinan sub-continent from 1959- 1960. Since then, it has conquered most of the epidemic areas (Wernsdorfer, 1994). Increment in the number of *Anopheles* species resistant to insecticides is equally on the increasing momentum. For instance, in 1946,

only two species of *Anopheles* were resistant to DDT, but from the two subsequent reports by 1991 and 1992 a total of 55 resistant *Anopheles* species have been recorded. Of these 21 were very important vectors of malaria. Nearly all members of *Anopheles gambiae* complex, that are the potent vectors of malaria in Tropical Africa, have shown various degrees of resistance to DDT as well as to other organo-chlorine insecticides (Gilles and Warrel, 1993). As an example, *Anopheles gambiae* s. s. in several West African countries has been reported as resistant to permethrin and with delayed knockdown (decreased susceptibility) to deltamethrin and lambda-cyhalothrin. In Indonesia, *Aedes aegypti* is reported as highly resistant to propoxur, bendiocarb and pyrethroids (WHO, 1996).

There are other factors contributing to this problem in addition to vector and malaria parasite resistances. Global warming is becoming one of the major causes for encroachment and occurrence of malaria epidemics in high altitude areas of Africa, including Ethiopia (Woyessa *et al.*, 2002). Meteorological variables are becoming highly conducive for mosquito breeding and survival through out the year, other epidemiological factors, and developmental activities (irrigation, resettlement) may have also contributed to the rapid increase in malaria incidence (Darrheim and Govere, 2002).

The disease is also an important impediment to economic and social development in most developing countries. It imposes very significant economic costs on some of the poorest nations. In 1997, it is estimated that the African region lost more than \$ 2 billion because of malaria and malaria-related diseases (MOH, 2002).

Therefore, malaria remains one of the major determinants of ill health in developing countries. Increased interest in tackling the malaria problem started towards the end of the 1980s, with the call for the African countries to evaluate their malaria control situation and reallocate adequate resources towards malaria control efforts. Research on malaria has been strengthened by World Health Organization and partners sensitized to participate in malaria control efforts in Africa. The Roll Back Malaria movement will

further efforts towards collaboration of all stakeholders for better planning and implementation of malaria control (MOH, 2002).

1.2 Epidemiology of malaria

1.2.1 Distribution

Malaria occurs as far north as 64⁰N latitude and as far south as 32⁰S latitude. And also in the Dead Sea area at 400m below sea level and in Londiani (Kenya) at 2600m above sea level and in Cochabamba (Bolivia) at 2800m above sea level (Gilles and Warrel, 1993). There are large areas free of malaria within these limits of latitude and altitude, since the transmission of malaria depends on local environmental conditions. Among the endemic tropical diseases occurring in epidemic form, malaria has a major place. Its repeated epidemics were reviewed elsewhere (Najera *et al.*, 1998).

1.2.2 Malaria parasites

There are four species of the malaria parasite causing serious disease in man: *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. falciparum*. *P. vivax* has the widest geographical range; it is prevalent in many temperate zones, but also in the subtropics and tropics. *P. falciparum* is the commonest species throughout the tropics and subtropics as well as in some areas with a temperate climate. *P. malariae* is patchily present over the same range as *P. falciparum* but much less common. *P. ovale* is found mainly in tropical Africa, but also occasionally in the west Pacific. *P. vivax* and *P. ovale* remain in the liver and enter dormant stage as long as 20 years (Martin and Lefebvre, 1995). But, *P. falciparum* and *P. malariae* do not persist in the liver, however in untreated or inadequately treated infections, erythrocytic parasites may persist from a month to years and produce clinical diseases.

1.2.3 Malaria vectors

Out of 430 different *Anopheles* species reported, about 70 of them transmit the human malaria (Martin and Lefebvre, 1995; Service, 2004). Anopheline vectors transmitting human malaria are divided as main and secondary vectors based on their role of transmission. *An. gambiae* complex are the main vectors and the *An. funestus* having a wide distribution and *An. nili* and *An. moucheti* with limited distribution *An. pharoensis*, *An. ziemanni*, *An. rufipes* and others are the secondary vectors in tropical Africa. Cytogenetic techniques revealed that the *An. gambiae* complex comprises six named and one unnamed sibling species namely *An. gambiae* s.s., *An. arabiensis*, *An. melas*, *An. merus*, *An. bwambae*, *An. quadriannulatus* species A and *An. quadriannulatus* species B (Hunt *et al.*, 1998; Coetzee *et al.*, 2000). *An. arabiensis*, and *An. gambiae* s.s. are the most important vectors of human malaria in sub-Saharan Africa (Coetzee *et al.*, 2000). The two species occur in sympatry in most parts of the continent. *An. gambiae* s.s. is dominant in the equatorial rain forest in the more humid areas. It is the most efficient of malaria vector, being anthropophilic and endophilic. It breeds in sunny temporary pools of water and the adult is present only in open places. Its density increases with the degradation of forest and thus linked with human activities (Gillies and Demellion, 1968).

An. arabiensis is more concentrated in the lower rainfall zones with annual rainfall less than 1000mm which represents the drier Savannah areas than the *An. gambiae* s.s. which is more concentrated in higher rainfall zones of greater than 1000mm (Coetzee *et al.*, 2000). *An. arabiensis* though predominant in dry areas and the Savannah belt, is also present in some degraded forest areas of central and West Africa. It is more abundant in the drier inland areas of east Africa and during the dry season. Its presence in the irrigated plains has been confirmed. Being also zoophilic compared to *An. gambiae* s.s., it is a less efficient vector. *An. arabiensis* is dominant in the Sudan Savannah and further north to the Egyptian border (Gillies and Demellion, 1968).

An. quadriannulatus species B is markedly exophilic and zoophagic and has negligible malaria vector capacity under natural conditions. Its endophilic tendency is reported in stables and dwellings in Ethiopia (White *et al.*, 1980; Janssens and Wery, 1987). In Uganda, *An. bwambae* is a very localized and a minor malaria vector which breeds in geothermal waters) (Gilles and Warrel, 1993). *An. melas* lives along the west coast of Africa, but spreads up to 100km inland. *An. merus* lives along the East (Indian Ocean) Coast counterpart, and penetrates inland as far as 220km. Both species have local importance in malaria transmission (Janssens and Wery, 1987).

An. funestus is capable of producing very high inoculation rates in a wide range of geographic, seasonal, and ecological conditions in Africa (Coluzzi, 1984). In Ethiopia, four members of the *An. funestus* group, which comprise seven species occur. These are *An. funestus*, *An. confuses*, *An. leesoni*, and *An. rivulorum* (Mekuria, 1983). It is found in a degraded forest and Savannah and on mountain slopes. It is potentially anthropophilic and endophilic vector with low sporozoite index. *An. pharoensis* is widely distributed in Africa and can transmit malaria actively even in the absence of the main malaria vector (Janssens and Wery, 1987).

An. nili and *An. moucheti* are both associated with running rivers, their larval stages find a suitable environment along the river banks. *An. nili* lives in the west and central African equatorial area, mainly in forest but occasionally in Sahel Savannah, and is more abundant during the rainy season. It needs fast running water and there are anthropophilic and zoophilic forms. *An. moucheti* occurs in the central African equatorial forest and the neighboring area, in more slowly running water with dense vegetation on river banks (Janssens and Wery, 1987; Gilles and Warrel, 1993).

An. pharoensis has a very wide distribution and occupies a broad variety of ecological zones except primary forest. It is a vector and can maintain active transmission of malaria in areas where the main vectors are absent (Janssens and Wery, 1987).

1.3 Malaria in Ethiopia

1.3.1 Distribution

Malaria is causing serious problems on health and socio-economic situation of the country (Tulu, 1993). Altitude and topographical and climatic factors determine the distribution of malaria in Ethiopia. Thus, malaria situation is unstable characteristically in higher altitudes, particularly above 1800m and like other sub-Saharan countries, Ethiopia suffers from both unstable epidemics in highlands and stable endemics in lowlands (Woube, 1997). 75% of the country is at risk of malaria, most of the lowland mass where more than 65% of the population live (Mitiku *et al.*, 2003; MOH, 2002). Ecological changes have immense roles in changing the epidemiology of malaria in the country. For instance, in Gambella, it was found that a degree of diverse malaria prevalence is associated with settlement schemes and land-use changes, unexpected rainfall patterns, temperature increase and poverty. As a result, the number of malaria affected people has increased (Woube, 1997). Studying the effect of agro-ecological change on malaria transmission in Bure in northwestern Ethiopia, Kebede *et al.* (2005) reported that there is direct relationship between percentage of maize cultivation, malaria outpatients and the number of incidence of malaria cases. Najera (1989) suggests another problem that tropical Africa and some areas of south-east Asia faced not only do they contain the highest levels of endemicity, but also have areas inhabited by populations with primitive tribal organizations incapable of supporting complex administrative structures and high costs of malaria eradication campaigns.

1.3.2 Malaria vectors in Ethiopia

The cornerstones for the knowledge of anopheline identification and distribution in the country were the works of Verrone (1962a & b), O'Connor (1967), and Gillies and De Meillon (1968). A total of 42 *Anopheles* species have been recorded. Two members of the *An. gambiae* complex, *An. arabiensis* and *An. quadriannulatus* species B occur in Ethiopia (White *et al.*, 1980; Hunt *et al.*, 1998), the former being the chief vector. Outside the *An. gambiae* complex, other anophelines which occur in Ethiopia are *An.*

funestus group, *An. pharoensis*, *An. nili* and etc. (O'connor, 1967). The available information about the present malaria vectors indicates that *An. arabiensis* is the major vector in the country (Ribeiro *et al.*, 1996; Abose *et al.*, 1998). It breeds in small, natural unshaded collections of water, such as produced by rain, temporary breeding places created by man are among most important to the species which permit both extensive and intensive development (O'connor, 1967). In studying the relationship between source reduction of mosquito larval habitat and malaria transmission in Tigray, Yohannes *et al.* (2005) reported that the major breeding habitats of *An. arabiensis* were areas of seepage at the dam base, leaking irrigation canals, pools that formed along the bed of streams from the dam and man-made pools.

An. funestus and *An. pharoensis* are considered to be secondary vectors of malaria next to *An. arabiensis* in Ethiopia (Mekuria,1983, Tulu, 1993). They breed in large, permanent and shaded water found in swamps, along lake shores and in irrigation ditches (O'connor, 1967). *An. nili* was identified as relatively more important vector than *An. arabiensis* in Gambella in much smaller villages around the river (Krafsur, 1970). It is the least common species and more localized to southwestern, western and northwestern parts of the country. Nigatu *et al.* (1994) reported that *An. gambiae* s.l. from the town of Gambella and *An. pharoensis* from Finkio (Baro Abol), in Gambella are vectors of malaria parasites, *P. falciparum* and *P. vivax* respectively. Ribeiro *et al.* (1996) studying temporal spatial distribution of anopheline mosquitoes in an Ethiopian village, Sille, 40km south of Arba-Minch in the Rift Valley region, reported that *An. gambiae* s.l. was the principal species followed by very low densities of *An. funestus*, *An. nili* and *An. pharoensis*.

1.3.3 Malaria parasites in Ethiopia

In Ethiopia, *P. falciparum* and *P. vivax* constitute 60% and around 40%, respectively. *P. malariae* comprises less than 1% and is mainly reported from Arba-Minch and from Nazareth, central Ethiopia. *P. ovale* is identified from a few patients who live or lived in Humera, Gambella, Arba-Minch (Gebre-Mariam, 1984; Tulu, 1993).

1.4 Malaria vector control in Ethiopia

1.4.1 Chemical control

Ethiopia initiated to launch National Malaria Eradication Service in 1967 (Gish, 1992). The strategy was to break the chain of transmission by spraying pesticides (DDT) that kill the mosquito vectors. It was conducted in areas of below 2000m elevations with DDT spray in human dwellings and chemotherapy to malaria cases and their families throughout the country. The strategy was not successful partly because of some technical and financial constraints in countries and institutions that were supporting the eradication effort (MOH, 2000).

In 1971, the Malaria Eradication Program was replaced by Malaria Control Program (Gish, 1992). This strategy was operating to prevent and control malaria with the concept of Primary Health Care. The main approach of the Control Program is delivering early diagnosis and prompt treatment of cases by antimalarial drugs, and selective and effective vector control as well as epidemic prevention and control. Indoor residual house spraying of a chlorinated insecticide, dichloro-diphenyl-trichloroethane (DDT) remained to be the major tool in malaria control that reduce the longevity of vector mosquitoes and in turn reduced the morbidity and mortality due to malaria (Fetene, 1997).

Larviciding which are used in selected regions has its own disadvantage. Larviciding is not effective in most rural areas of the African region where breeding sites are ubiquitous and short-lived requiring frequent applications. Larvicides are harmful to other non-target organisms including natural predators of mosquito larvae and may result in environmental pollution if insecticides to be used are not well selected, and larvicides are expensive (MOH, 2002). Insecticide resistance by the vector mosquitoes and antimalarial drug resistance by the malaria parasites reported in different parts of Ethiopia are becoming the problem in Control Program (WHO, 2001). The problem of resistance to insecticides and the evasive behavior of vectors became more widespread and evolved more rapidly than expected. Parasite resistance to antimalarial drugs was also recognized

(WHO, 1996), although during the 1960s resistance to chloroquine seemed to be restricted to northern parts of South America and Indochina peninsula (Najera, 1989). Some degree of DDT resistance has been detected in Gambella (Nigatu *et al.*, 1994; Abose *et al.*, 1998), in the Awash Valley (Ameneshewa and Service, 1996), in Zeway areas (Abose *et al.*, 1998), and Melka-worer and Metehara not only to DDT but also 25% of the vector (*An. arabiensis*) was found to be resistant to permethrin in Metehara (Balkew *et al.*, 2003).

1.4.2 Environmental management

Environmental management refers to planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors with a view to preventing or minimizing vector propagation and reducing man-vector-pathogen contact (WHO, 1982). Environmental modification is any physical transformation that is permanent or long-lasting of land, water and vegetation, aimed at preventing, eliminating or reducing the habitats of vectors without causing adverse effects on the quality of human environment. It includes drainage, filling, land leveling and transformation and impoundment margins (WHO, 1982; Yohannes *et al.*, 2005).

Yohannes *et al.* (2005) reported that source reduction was carried out by community resulting in 49% relative reduction in *An. arabiensis* adults in the dam village in Tigray compared with pre-intervention period. However, prevention of breeding in and around dwellings has little effect on malaria transmission except when widely applied.

1.4.3 Biological control

Biological methods of mosquito control basically consist in the utilization of natural enemies (predators, parasites or pathogens) of mosquitoes and of biological toxoids to achieve an effective control (WHO, 1982; Yap, 1985). Many field trials have been carried out against vectors with each of these agents. A fish *Gambusia affinis* shows a

marked preference for mosquito larvae (El Safi *et al.*, 1985; Rozendaal, 1997). It is a voracious eater of mosquito larvae and, if introduced in sufficient numbers in pools, ponds and marshes, it can destroy large quantities of mosquito eggs, larvae and pupae (WHO, 1982; Rozendaal, 1997). Other fishes used are *Poecilia reticulata*, *Aplocheilus* species, *Aphanius* and *Tilapia species* (El Safi *et al.*, 1985; Yap, 1985; Lardeux *et al.*, 2002; Rozendaal, 1997). WHO (1996) reported that only fish predators (*Gambusia affinis*) of mosquito larvae have been successfully and extensively applied in some areas. Predacious mosquito larvae of *Toxorhynchites* species have been found to be promising and effective for the control of larvae of some mosquito species in plant exils, tree holes, cut bamboos, abandoned containers and similar sites (WHO, 1982). However, there are some problems associated: importation of predatory fish into any location has serious and complicated environmental impact, and mass production still remains as a major obstacle in the development of predaceous mosquitoes as biological control agents (Yap, 1985).

Biological control methods have the advantage of target specificity with little effect on non-target organisms and regarded environmentally safe. However, the high cost and difficulties encountered in mass production, preclude their wide use, especially in tropical countries. Among the bacteria, *Bacillus thuringiensis* H-14 (*Bti*) and *B. sphaericus* have shown high potency for mosquito control without adversely affecting non-target organisms and the environment (Rozendaal, 1997; Saitoh *et al.*, 1998; Saringer *et al.*, 1998; Lardeux *et al.*, 2002). Among the fungi the imperfect fungi of the genera *Culicinomyces*, *Tolypocladium*, *Metarhizium* and *Beauveria* can be readily cultured on artificial media to yield infective stages of mosquito control. Two most known species of protozoa are *Vavraia culicis* which parasitized mainly on culicines, and *Nosemia algerae* which appeared to be most effective against *Anopheles* (Yap, 1985).

1.4.4 Personal protection methods

1.4.4.1 Insecticide treated mosquito nets

Insecticide-treated mosquito nets (ITNs) have become the favoured vector control tool for malaria and other vector-borne diseases, such as leishmaniasis. ITN implementation ranges from humanitarian emergency relief to use as a component of national malaria control programmes in stable situations (Kolaczinski and Graham, 2004). In different parts of the world, renewed efforts aimed at disease prevention through ITNs and synthetic repellents for personal protection against biting mosquitoes had also been taken place (Guyatt and Snow, 2004). ITNs can substantially lower morbidity and mortality and remain the most commonly advocated methods for malaria prevention. ITNs have revitalized interest in vector control of malaria in sub-Saharan Africa where high transmission levels result in extremely stable malaria prevalence, incidence and clinical burden. ITNs protect their occupants by diverting host seeking vectors to look for a blood meal elsewhere and by killing those that attempt to feed (Killen *et al.*, 2002).

In Ethiopia, the use of ITNs was introduced in selected areas as one of the important malaria control measures in 1997/98 on cost recovery basis (MOH, 2000). But there are disadvantages: in hot climates, nets may be uncomfortable to use due to poor ventilation, cost of nets precludes wide use in many rural poor communities, frequent washing of the nets reduces the effectiveness of the treated nets. If people are not used to them, it might require behavioral change and people need to be taught how best to use the nets (MOH, 2002; Kolaczinski and Graham, 2004). Another problem to the use of ITNs is that it is used at bed time but, most people are bitten outdoors before bedtime.

1.4.4.2 Insect repellents and their Mode of action

Chemicals play an important role in influencing insect vital activities (finding food, opposite sex, an oviposition medium). Receptors (chemoreceptors) of insects that are sensitive to chemicals are located on several strategic parts of the body. The antennae bear olfactory receptors and contact chemoreceptors (Schoonhoven, 1977; Gibson and Torr, 1999). Blood sucking insects are attracted to human body odors, which have different substrates (urine, feces, carbon dioxide) (Rebollar-Tellez, 2005). These odors are important as long-range and short-range cues for host recognition even though the exact relationship between human body odors and the attractiveness of blood-sucking insects remains unknown (Rebollar-Tellez, 2005). Gibson and Torr (1999) reported that carbon dioxide appears to be universally attractive to mosquitoes, and is probably well-understood of the volatile host cues. Rebollar-Tellez (2005) reported that body odors are actually by-products of the decomposition of odorless skin chemicals, and that the final attraction is due to the balance between attractive and non-attractive fractions.

Many potential repellents can be considered as barrier to the insect, preventing either landing or penetration of the skin. Repellents contain volatile compounds that rely on vapor pressure and temperature to release specific chemicals that insects avoid (Novak and Gerberg, 2005). The mode of action of repellents is not actually to repel biting insects but seem to be to cancel out the attractant properties of the animal body or cancel the signals of attraction of animals (Davis, 1985). Dogan *et al.* (1999) reported that in the absence of a host, DEET was an attractant and in the presence of a host, it was an inhibitor of attraction in an experiment using the mosquito *Ae. aegypti* in a novel olfactometer that measures movement towards and away from a stimulus. Inhibition occurred in the gaseous phase and was therefore not the result of the physical properties of DEET, and lactic acid, a component of human sweat that is an attractant to mosquitoes, is the target of this inhibition (Dogan *et al.*, 1999). Kline *et al.* (2003) reported similar result to Dogan *et al.* (1999) that in the absence of human attractant mixtures, DEET, linalool and dehydrolinalool resulted in attraction and/or orientation of mosquitoes to the chemical source, and in the presence of human attractant mixtures, activation and/or orientation of mosquitoes to each of the three chemicals was reduced,

implying that DEET did not manifest repellency. Therefore it is assumed that repellents act by blocking receptors on the hairs of mosquito antennae that detect body heat, moisture and carbon dioxide, causing disorientation and loss of targeting ability (Burfield and Reekle, 2005).

Insect repellents are applied in various forms: directly to the skin, to clothing or other fabrics. They are recommended for people standing or sleeping out doors at night for work or leisure and those working during day time (Fradin, 1998). There are two kinds of repellents: synthetic and natural (plant-derived) repellents.

1.4.4.2.1 Synthetic repellents

There are many types of synthetic repellents so far manufactured. DEET (N,N-diethyl-m-toluamide) is the active ingredient of most commercial synthetic repellent formulations which are normally applied directly to skin or clothing e.g. arm and ankle bands, or mosquito screens. Its efficacy and low toxicity have been proved over many decades of widespread consumer use (Curtis *et al.*, 1987; Frances and Wirtz, 2005). There are also several synthetic repellents used in addition to DEET such as CIC-4 (2-hydroxymethylcyclohexyl), AI3-37220 (1-(3-cyclohexen-1-yl-carbonyl)-2-methylpiperidine) which are reported to provide >95% protection for 5 hrs after application (Frances *et al.*, 1998). KBR-3023 (a propidine compound) is another repellent which is found to be equally effective with DEET (Yap *et al.*, 1998). Bio-SkincareTM (BSC, oils of coconut, jojoba, rapeseed and vitamin E) and MosiguardTM towelletes with 0.57g quwenling are commercial repellents of *An. arabiensis*, the predominant vector of malaria in South Africa (Govere *et al.*, 2000). 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). Other disadvantages are associated with the use of DEET in 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). There have been also concerns over the toxicity of DEET. It irritates the eyes and mucous membrane when applied on the face (Osimitz and Grothaus, 1995).

1.4.4.2.2 Plant-derived repellents

In different parts of the world, recently research focuses on plant derived repellents for mosquito and other vector control. Directly the plant parts or their extracts or their essential oils are used as repellents. In Kenya, Seyoum *et al.* (2002) reported that *Eucalyptus citriodora* was the most effective repellent plant by thermal expulsion and direct burning of the leaves against *An. gambiae*. In Western Kenya, *E. citriodora* showed the highest repellency compared with *Ocimum* species during application of plant material by thermal expulsion (Seyoum *et al.*, 2003). Smoke of the leaves of lemon eucalyptus (*E. citriodora*) showed greater repellency on mosquito (Palsson and Jaenson, 1999). In Kenya, the seeds and leaves of *O. suave* were the most effective repellent (53.1%, $P < 0.0001$) against the main vector of malaria in Africa by thermal expulsion (Seyoum *et al.*, 2002; 2003). The leaves of *Lippia adoensis* are used in Ethiopian traditional medicine for the treatment of various kinds of skin diseases (Asres and Bucar, 2002). In Zimbabwe, 50% of the study subjects use *L. jaunica* as mosquito repellent by smoking and placing fresh plant parts (Lukwa *et al.*, 1999). In Kenya, *L. ukambensis* was tested in live, intact potted form and showed considerable repellency (45.2%) against *An. gambiae* s. l. (Seyoum *et al.*, 2003). Recently, Atsebeha (2005) studied the effects of thermal expulsion on burning of seeds and leaves of some aromatic plants in Koka area (*Schinus molle*, *Ocimum lamiifolium*, *Lantana camara*, *Pychnostachys abyssinica*, *Eucalyptus globulus* and *Melia azadarach*) and found some of them (*S. molle*, *O. lamiifolium* and *P. abyssinica*) significantly repelled endophagic mosquitoes (*An. arabiensis*, *An. phareonsis* and culicines) . However, the essential oils of these plants have not been evaluated in Ethiopia against mosquitoes and other biting insects.

Ali-Emmanual *et al.* (2003) reported that extracts of medicinal plants (*Senna alata*, *Lantana camara*, *Mitracarpus scaber*) in ointment form acted as fly repellents so that preventing the treated areas from suffering any other infections. In Thailand, the ethanol extract of *Curcuma aromatica* showed repellency against mosquitoes under laboratory

and field conditions (Pitasawat *et al.*, 2003). Quwenling, a repellent made from the waste distillate after extraction of oil from lemon Eucalyptus (*E. citriodora*) gave complete protection against *An. gambiae* and *An. funestus* for about 6-7 hours depending up on the formulation type in Tanzania (Trigg, 1996; Novok and Gerberg, 2005). In the laboratory tests, ethyl acetate extracts of *Hyptis suaveolens* Poit., *H. harmaja* L., *Rhododendron tomentosum* (Stokes) and *Myrica gale* L. significantly reduced probing activity of *Aedes aegypti* (Jaenson *et al.*, 2006). In the field in mixed spruce-deciduous woodland at Jularp, Hoor, Skane, in southern Sweden, extracts of leaves of *R. tomentosum*, *M. gale* and *Achillea millefolium* significantly reduced biting by *Aedes* mosquitoes (Jaenson *et al.*, 2006). Crude seed extract of celery, *Apium graveolens* L. showed repellency against *Ae. aegypti* adult females with ED₅₀ and ED₉₅ values of 2.03 and 28.12 mg/cm², respectively. It also provided biting protection time of 3 hours when applied at a concentration of 25gm% and can be considered as a probable source of some biologically active compounds used in the development of mosquito control agents (Choochote *et al.*, 2004). Metanolic extract of the leaves of *Ferronia elephantum* (Rutaceae) on *Ae. aegypti*, and the flower of *Lantana camara* (Verbenaceae) with coconut oil on *Ae. aegypti* and *Ae. albopictus* have repellent effects (Mittal and Subbarao, 2003).

Essential oils from various plants exhibited greater repellency on different insects. Oil from neem (*Azadirachta indica* A. Juss) was reported to have a good repellency action against mosquitoes (Sharma *et al.*, 1993). The essential oil from fresh leaves of *S. molle* isolated by hydrodistillation was tested for antibacterial activity and for antifungal activity and results showed significant activity against bacteria and fungi (Gundidza, 1993). Essential oils extracted from citronella grass (*Cymbopogon nardus* Randle) and hairy basil (*Ocimum americanum* L.) are potential repellents of mosquitoes (both oils provided 100% repellency 6 hours post application against *Culex quinquefasciatus*) that could potentially replace DEET, the most common chemical repellent currently available (Tawatsin *et al.*, 2001). *Ocimum selloi* oil is an effective mosquito repellent that presents a low toxicity, poses no mutagenic risk and seems not to be irritating to human skin (Padilha de Paula *et al.*, 2003). Essential oils from seeds of *O. suave*, *O. kilimandscharicum* and *O. kenyense* have also provided good protection against stored

product insect pests (Jembere *et al.*, 1995; Bekele *et al.*, 1996; 1997). Barnard and Xue (2004) reported that Soya bean oil prevented biting mosquitoes for ≥ 7 hours in the laboratory. However, none of the essential oils of *Ocimum* species, *Schinus molle*, *Eucalyptus* species and other aromatic plants found in Ethiopia have been evaluated for their repellency against mosquitoes or any other biting insects.

The existing malaria and vector control measures (reviewed above) are not sufficient. Unless alternative control strategies are developed, death and illness due to malaria will increase, and the diseases will continue to be a substantial barrier to the economic and social development of malaria endemic regions as well as a threat to the millions of people who travel to those regions each year.

Nowadays, the search for alternative, simple and cost effective tools for malaria vector control is underway in different parts of the world. One field of research interest is the search for new repellents, either synthetic or plant derived repellents. Plant based repellents that reduce man-vector contact have the advantage that they can be produced locally, thus reducing cost and, environmentally safe. It is based on this point of view that this study was conducted in the laboratory to evaluate the repellency of the essential oils of the following aromatic plants found in Ethiopia against *An. arabiensis* and *Ae. aegypti*.

1.5 Description of the test plants

1.5.1 *Eucalyptus citriodora*

Eucalyptus citriodora Hook. (Myrtaceae) is commonly referred to as a lemon scented gum, a straight, moderately tall tree with a smooth and deciduous bark and grayish in color. Its juvenile leaves are opposite, fairly broad, tapered at both ends and rough to the touch. Mature leaves are alternate, smooth, fairly narrow and tapered at both ends, 10-16cm long and 1-2cm broad; strongly lemon scented when crushed (Lassak and McCarthy, 1983). The plant grows in a wide range of climates between 1300 and 2400m

where rainfall is more than 900mm a year. It is a native of Queensland, Australia, and nowadays widely planted in subtropical and tropical Africa, Asia, South America, Oceania and temperate southern Europe (Fichtl and Adi, 1994). In Ethiopia it grows well in the compounds of the Essential oil Research Laboratory, Wondogenet in southern Ethiopia. It is widely planted but only successful in places with deep soil and rainfall over 1000mm (Friis, 1995).

Due to synergism between citronellol and citronellal present in the essential oil, it appears to have bacteriostatic activity towards *Staphylococcus aureus*. The kino (exudates of the bark) also contains the antibiotic substance citriodorol (Lassak and McCarthy, 1983). In Kenya, Seyoum *et al.* (2002) reported that *Eucalyptus citriodora* was the most effective repellent plant by thermal expulsion and direct burning against *Anopheles gambiae*. Repellent made from extraction of oil from lemon Eucalyptus showed comparable efficacy and duration of protection comparable to DEET (Trigg, 1996). Smoke of the leaves of lemon Eucalyptus showed greater repellency to mosquitoes (Palsson and Jaenson, 1999). Also in western Kenya, *Eucalyptus citriodora* showed the highest repellency compared with *Ocimum* species during application of plant material by thermal expulsion (Seyoum *et al.*, 2003). In the laboratory, 20% *E. citriodora* oil gave protection times and percentages of repellency of 150 minutes and 59.4%, 480 minutes and 52.4% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus*, respectively (Amer and Mehlhorn, 2006). But, its essential oil efficacy as a repellent against biting insects in Ethiopia has not been evaluated.

1.5.2 *Ocimum suave*

Ocimum suave Willd. (Lamiaceae) is a perennial herb or shrub, often densely and profusely branched up to 3m high with hairy opposite leaves. Flowers are small, white, sweet-scented, found in whorls in spikes (Fichtl and Adi, 1994). Active constituents may be phenolic compounds such as eugenol (Lassak and McCarthy, 1983). The plant is widely cultivated in home gardens and is common on shallow soils, evergreen bush land between 600-2200m. The leaves and flowers are used as local medicine against

headaches and skin problems and the sap against eye disease and typhus (Fichtl and Adi, 1994). Materials from the leaves and succulent stems of *O. suave*, provided effective repellency against stored product insect pests (Bekele *et al.*, 1996). In Kenya, Seyoum *et al.* (2002) reported that the leaves and seeds of *O. suave* were the most effective repellent (53.1% $P < 0.0001$) against *Anopheles gambiae* Giles. The strongly scented leaves of *O. suave* act as disinfectant and insecticide (Kokwaro, 1976). Thermal expulsion of *O. suave* showed significant repellency against the main vectors of malaria in Africa (Seyoum *et al.*, 2003). Seyoum (2003) reported that essential oils from the seeds and leaves of *O. suave* provided repellency against *An. gambiae* s.s for less than one hour complete protection in the laboratory in Kenya. However, the essential oils of *O. suave* have not been evaluated against biting insects in Ethiopia.

1.5.3 *Ocimum lamiifolium*

O. lamiifolium Hochst. ex Benth. (Lamiaceae) is an erect, robust branching shrub or herb growing to 3m tall. Leaves are ovate and opposite and flowers are pinkish in racemes. It grows beside roads and streams, in bush land and at forest edged and on grassland between 1200-2900m. Its leaves are used against eye diseases and head aches (Fichtl and Adi, 1994). *Ocimum* species and their essential oils have been traditionally used to kill or repel insects, and also to flavor foods and oral products, in fragrance, in folk medicine and as condiments (Padilha de Paula, 2003). Studying the use of medicinal plants in self-care in rural central Ethiopia by Gedif and Hahn (2003) reported that out of 25 plant species belonging to 21 families had medicinal values and *O. lamiifolium* is mentioned among the most frequently used plants. Thermal expulsion of *Ocimum*. species showed significant repellency against the main vectors of malaria in Africa (Seyoum *et al.*, 2003). The repellent activity of fresh *O. canum* showed 63.6% reducing mosquito biting activity in Kenya (Palsson and Jaenson, 1999). In Brazil, *O. selloi* oil has been found to be effective mosquito (*An. braziliensis*) repellent in the field (Padilha de Paula *et al.*, 2003). The oils of *Ocimum* species shows remarkable repellency to mosquitoes and stored product insect pests. Materials from the leaves and succulent stems *O. kilimandscharicum* and *O. kenyense* provided effective repellency against stored product insect pests (

Bekele *et al.*, 1995; 1997). Atsebha (2005) investigated the repellency of *O. lamiifolium* by thermal expulsion and direct burning of the leaves. However, the essential oils of *O. lamiifolium* have not been evaluated against biting insects in Ethiopia.

1.5.4 *Schinus molle*

Schinus molle L. (Anacardiaceae) locally referred to as “true-man-tree” is a pepper tree which is frequently grown as an ornamental in Mediterranean region. Its original habitat ranged from southern Brazil to Chile and Mexico, also comes from the New World. It is a medium to small shrubby tree growing 4-8m in height with a trunk 25-35cm in diameter. It has long, pinnate leaves and inconspicuous greenish white flowers that develop in to pretty, pea-green drupes in long, hanging infructescence (fruiting stage of the inflorescence) (de Wit, 1993). In Ethiopia, the plant is widely grown through out the country from Afar lowlands to 2400m (Fichtl and Adi, 1994) as ornamental and shade tree especially in the towns and cities of the Rift valley in Ethiopia. Its branches are commonly placed on dining tables in hotels, restaurants and resident places to repel flies in some parts. As a repellent against biting insects it has not been evaluated. Dikshit *et al.* (1986) reported that the essential oil of *S. molle* comprising 50 constituents exhibited the maximum fungitoxic activity during the screening of some essential oils against some common storage and animal pathogenic fungi.. Gundidza (1993) reported that the essential oil from the fresh leaves of *S. molle* isolated by hydro-distillation exhibited significant antibacterial and antifungal activity against bacteria and fungi. Atsebha (2005) has shown that thermal expulsion and direct burning of its leaves and seeds exhibited some degree of repellency against mosquitoes. However, the essential oils of *S. molle* have not been evaluated as repellents against mosquitoes and other biting insects in Ethiopia and elsewhere.

1.5.5 *Lippia adoensis*

The genus *Lippia* (Verbenaceae) consists of approximately 200 species of herbs, shrubs and small trees (Tereblanche and Kornelius, 1996). In Ethiopia, *Lippia* is represented by only five species of which *Lippia adoensis* Hochst. ex Walp. is the most important in

terms of its use in traditional medicine and as a condiment. Two varieties of *Lippia adoensis* namely, variety *adoensis* (wild variety) and variety *koseret* (cultivated variety) have been described in Ethiopia (Demissew, 1993). The leaves of the former have strong and pungent scent, and the plants are endemic to the high lands of Ethiopia where they grow between altitudes of 1600-3000m above sea level. The leaves of the cultivated variety, on the other hand, have sweet scent and the plants are known to grow in the Shoa administrative region in altitudes ranging between 2000-2700m above sea level. The wild variety is used to impart fresh and spicy fragrance. Moreover, the leaves of this species are used in Ethiopian traditional medicine for the treatment of various kinds of skin diseases. The cultivated variety has not been reported for its medicinal uses (Asres and Bucar, 2002). The leaves and flowers of some species of *Lippia* are first rubbed between the palms to get the maximum scent emitted, and when sniffed the subject is set sneezing which then clears the nose. For the treatment of malaria, a decoction of boiled leaves is taken and the whole body bathed in the same fluid. Pounded leaves can also be applied on cut wounds, or soaked in water and the juice drunk for the treatment of tape worm and for indigestion (Kokwaro, 1976). In Ethiopia, the cultivated *Lippia adoensis* is traditionally used to flavor butter for a special dish known as “kitfo” (Abegaz *et al.*, 1993). In Zimbabwe, Lukwa *et al.* (1999) reported that 50% of the study subjects use *L. jaunica* as mosquito repellent by smoking and placing fresh plant parts. In Western Kenya, *L. ukambensis* was tested in live, intact potted form and showed significant repellency (45.2%) against *An.gambiae* s. l. (Seyoum *et al.*, 2003). However, their essential oils have not been tested for their repellency against biting insects in Ethiopia.

1.5.6 *Eucalyptus globulus*

Eucalyptus globulus, (nech bahirzaf in Amharic) is a long tree up to 55m tall and trunk attaining almost 2m, native to Australia, but widely distributed in many countries including Ethiopia. Blue gum is the most widespread eucalyptus in Ethiopia, now grown in plantations between 1800-3200m through out the country where rainfall ranges 800-1500mm per year (Fichtl and Adi, 1994). About 100 species of *Eucalyptus* trees are recorded in east Africa. *E. globulus* is cultivated in Ethiopia for timber production (Friis, 1995).

The essential oil of *E. globulus* was investigated in Thailand for its repellent activity against mosquitoes on hairless mice and found to be active repellent (Choi *et al.*, 2002). In the laboratory condition, in China, Yang and Ma (2005) reported that 15% concentration of the essential oil of *E. globulus* gave protection to humans for at least three hours against *Ae. albopictus*. In Germany, Amer and Mehlhorn (2006) reported that protection times and percentages of repellency for 20% *E. globulus* oil were 60 minutes and 57.6%, 330 minutes and 52.4% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus*, respectively. Although fresh branches of *E. globulus* are traditionally and widely used to chase away intruding armies of ants and for treatment of common cold and cough, its role as repellent against biting insects has not been evaluated in the country.

2 OBJECTIVES OF THE STUDY

2.1 General objectives

To evaluate repellency effects of essential oils of some aromatic and potential repellent plants against laboratory colonies of *An. arabiensis* and *Aedes aegypti*.

2.2 Specific objectives

1. To evaluate percentage repellency of essential oils of some local plants against laboratory colonies of *An. arabiensis* and *Aedes aegypti*.
2. To evaluate duration of protection of essential oils of some aromatic plants against laboratory colonies of *An. arabiensis* and *Aedes aegypti*.
3. To evaluate blend effects of repellent oils of the different test plants against *An. arabiensis* and *Aedes aegypti*.

3 MATERIALS AND METHODS

3.1 Selection and collection of the test plants

Six plant species: *Ocimum suave*, *O. lamiifolium*, *Eucalyptus citriodora*, *E. globulus*, *Lippia adoensis* and *Schinus molle* (Annex 24) were selected based on taxonomic relationships to known repellent plants, lack of information on varieties found in Ethiopia, their aromatic properties of the plant, traditional use of local plants as repellent for some insect pests (ants) and literature information. *O. suave* was collected from around Ghibe River, south western Ethiopia, *O. lamiifolium* was collected from Hossana, southern Ethiopia, *L. adoensis* was collected from Entoto Mountain, Addis Ababa, and *S. molle* was collected from around the high way on the Debre-Zeit road. The essential oils of *E. citriodora* and *E. globulus* were given free of charge from the Essential Oil Research Center, Addis Ababa. Taxonomy of all these plants were verified and confirmed by prof. Sebsibe Demissew and Dr. Ensermu Kelbessa (Biol. Department, AAU).

3.2 Essential oil extraction

After collection of the test plants from their natural habitats within the country, their essential oils were extracted by steam distillation by the staff of the Essential Oil Research Center, Addis Ababa. In this process, the leaves were cut into small pieces and placed into a distillation flask with clavenger apparatus (Annex 25) and with much water. The distillation chamber was heated at about 120⁰c and allowed to boil until the distillation process was completed. The distillate was then collected from the separating funnel in which the aqueous portion was separated from the essential oil. The water layer was slowly drawn off until only the oil layer remained. The oil was collected in a cylinder and stored at about 4⁰c until it was tested for mosquito repellency.

The leaves of *O. lamiifolium* and *L. adoensis* were first carefully collected and dried under shade and placed into a distillation flask. Fresh leaves of *O. suave* and *S. molle* were cut into pieces and placed into a distillation flask for the isolation of essential oil.

3.3 Test procedure

The methods described by Frances *et al.* (1998) and Seyoum (2003) were used. Tests were conducted in a room using cages (30cm× 30cm× 30cm) (Curtis *et al.*, 1987) which were prepared locally (Annex 26). Test mosquitoes were laboratory colonies of *An. arabiensis* and *Aedes aegypti* maintained in the Aklilu Lemma Institute of Pathobiology. The temperature and relative humidity of the test room during tests were about 20 -25⁰c and 60 -70 % during the tests. Tests were conducted during the day in the dark room and counting of mosquitoes landing/probing was undergone by using torch batteries.

3.3.1 Dose response tests

Fifty nuliparous females (3-6 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 was recorded (to ensure mosquitoes were avid). 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 cage after repellent oil formulation was applied evenly between the wrist and elbow and dried for 2 minutes (Annex 18). The fore-arm was treated with acetone solutions of test samples (0.5-0.6ml based on surface area of the volunteers) at a rate of 0.001ml/cm².

The surface area of the forearm was calculated using the following formula:-

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

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 (Frances *et al.*, 1998).

In this study three adult male volunteers were involved. The arms were first cleaned with water and 95% ethanol, and air-dried before application of the test oils and control acetone. A surgical glove was worn on the forearm (wrist and fingers) during each test to prevent biting on the untreated arm. Each test consisted of two parts where each volunteer used the right arm for treatment and the left arm for control. Separate cages were used for control and treated arms to avoid contamination of the control cage with oil. The exposure time in the cage was 30 seconds, and the number of mosquitoes that landed or probed the arm were recorded for each subject.

The dose response tests were conducted on series of concentrations (1% to 10%) of the repellent and untreated control. The concentrations were tested one after the other (from the lowest to the highest) to the caged mosquitoes for a particular repellent until no landing/probing was recorded at a given concentration of the repellent oil (1%-10%). 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 as described below.

3.3.2 Evaluation of protection time

Protection time of the essential oils of all candidate plants was determined at 10% and 20% concentrations. Formulated commercial DEET was used as a standard for comparison of protection time to that of the candidate essential oils. It is a common

standard against which the protection time of repellents is compared in many laboratories (Collins and Brady, 1993; Govere *et al.*, 2000; Seyoum, 2003). The control acetone and test oils applied on the fore-arms were tested in similar way described in dose response test above. After application of repellents in between the test periods, subjects were instructed not to rub, touch, or wet the treated arms and were restricted to stay in a room to minimize the loss of repellent effects of oils on treated arms. Tests were undertaken immediately after application of repellents followed by 1, 2, 3, 4, 5, 6, 7 and 8 hours post-application, and the experiment was only stopped when no significant protection was observed by the essential oils.

Duration of protection time for blend effects of 15 combinations of the 6 essential oils at 10% and 20% concentrations was also tested against *An. arabiensis*. The combinations were made by mixing equal concentrations of oils in 1:1 (v/v) ratio. For example, to make 2ml of 10% concentration of *O. suave* + *L. adoensis*, 0.1ml from 100% concentration of *O. suave* was mixed with 0.1ml from 100% concentration of *L. adoensis* and this mixture that is 0.2ml was diluted by 1.8ml of acetone. The formula, % concentration (C_2) = C_1 (100% concentration of the oil) \times V_1 (volume of the oil to be mixed with acetone)/ V_2 (final volume of the test oil) was used for calculation to prepare test concentrations.

3.3.3 Data analyses

The totals of each hour count of each replicate test (oils and controls) were summed and percentage of repellency reduction was calculated by comparing the number of bites for control against the number of bites for repellent treated volunteers. The formula used to calculate this is

$$\text{- \% repellency} = (C-T/C)(100), \text{ where}$$

C = number of mosquitoes counted from control and

T = number of mosquitoes counted from treated (Yap *et al.*, 1998). This was calculated for each of the concentrations of the test oils in dose response studies (Seyoum, 2003).

The median effective dosage (ED₅₀) and ED₉₅ of the oils were determined using computer software for probit analysis (Finny, 1971) by SPSS 11.0 for windows. Effectiveness of the test oils was determined by comparing the 95% confidence intervals of the ED₅₀ values.

To evaluate and compare the efficacy of each mosquito repellents in each of the above experiments, appropriate statistical method, one way ANOVA was used. Significant difference between the means was separated using Tukey's honestly significant difference (HSD) test (Barnard and Xue, 2004).

4 RESULTS

4.1 Dose response studies of the essential oils

Table 1 shows the repellency effects of essential oils of different plants tested against *An. arabiensis* in the present dose response studies. The essential oils of all tested plant species showed high level of repellency with ED₅₀ values ranging from 1.476 (95% CI = 0.921-1.882) for *L. adoensis* to 4.824 (95% CI = 4.291-5.385) for *O. lamiifolium* and ED₉₅ values ranging from 4.999 (95% CI = 3.866-5.529) for *L. adoensis* to 9.993 (95% CI = 8.700-12.216) for *E. globulus*. The next essential oil that possessed high level of repellent activity after *L. adoensis* with the lowest ED₅₀ value was that of *O. suave* with ED₅₀ and ED₉₅ values of 1.699 (95% CI = 0.822-2.338) and 6.737 (95% CI = 5.669-8.619) respectively. *E. citriodora* followed *O. suave*, *E. globulus* followed *E. citriodora*, *S. molle* followed *E. globulus* and *O. lamiifolium* followed *S. molle*. *O. suave* and *L. adoensis* have overlapping 95% confidence intervals of their ED₅₀ values. *E. citriodora*, *E. globulus*, *S. molle* and *O. lamiifolium* have overlapping 95% confidence intervals of their ED₅₀ values. *L. adoensis* and *O. suave* have non overlapping 95% confidence intervals, where as the other four oils have overlapping 95% confidence intervals at ED₉₅ values.

Table 2 summarizes the repellency effects of essential oils of the test plants against *Ae. aegypti* in the present dose response studies. Similarly all essential oils showed high level of repellency. However, *O. suave* showed the highest level of repellent activity with ED₅₀ and ED₉₅ values of 1.508 (95% CI = 0.630-2.133) and 6.273 (95% CI = 5.275-8.029) respectively against *Ae. aegypti*. The repellent activity of *E. citriodora* was the least among the test plants against *Ae. aegypti* with the highest ED₅₀ and ED₉₅ values. When put in descending order of their effectiveness, *O. suave* came the first, *S. molle* the second, *L. adoensis* the third, *E. globulus* the fourth, *O. lamiifolium* the fifth and *E. citriodora* the last. The confidence intervals of ED₅₀ values of *O. suave*, *S. molle* and *L. adoensis* overlapped and *E. citriodora*, *E. globulus*, *O. lamiifolium* and *L. adoensis* overlapped. However, the confidence intervals of ED₉₅ values of all the six test oils almost overlapped indicating that their effectiveness is the same at ED₉₅ values.

Table 1 The ED₅₀ and ED₉₅ values of essential oils of plants tested for repellency effect against adults of *Anopheles arabiensis* in laboratory dose response tests

Plant species	ED ₅₀ (95% CI)	ED ₉₅ (95% CI)	X ² (df=2)	P-value
<i>L. adoensis</i>	1.476 (0.921-1.882)	4.499 (3.886-5.529)	4.328	0.115
<i>O. suave</i>	1.699 (0.822- 2.338)	6.737 (5.669- 8.619)	5.854	0.054
<i>E. citriodora</i>	3.330 (2.364- 4.043)	8.662 (7.571- 10.500)	5.611	0.060
<i>E. globulus</i>	3.904 (2.923- 4.656)	9.993 (8.700- 12.216)	5.674	0.059
<i>S. molle</i>	4.145 (3.432- 4.746)	8.763 (7.800- 10.288)	1.794	0.408
<i>O. lamiifolium</i>	4.824 (4.219- 5.385)	9.128 (8.198- 10.562)	3.531	0.171

Table 2 The ED₅₀ and ED₉₅ values of essential oils of plants tested for repellency effect against adults of *Aedes aegypti* in laboratory dose response tests

Plant species	ED ₅₀ (95% CI)	ED ₉₅ (95% CI)	X ² (df=2)	P-value
<i>O. suave</i>	1.508 (0.630- 2.133)	6.273 (5.275- 8.029)	4.931	0.085
<i>S. molle</i>	2.534 (1.272- 3.357)	8.072 (6.976- 9.992)	1.554	0.460
<i>L. adoensis</i>	2.681 (-3.924- 6.605)	6.353 (4.174- 55.856)	6.099	0.047
<i>E. globulus</i>	4.092 (3.406- 4.678)	8.540 (7.604- 10.017)	2.755	0.252
<i>O. lamiifolium</i>	4.212 (3.587- 4.762)	8.286 (7.421- 9.629)	1.376	0.503
<i>E. citriodora</i>	4.806 (4.176- 5.387)	9.332 (8.355- 10.851)	5.226	0.073

4.2 Duration of protection by the essential oils

Figures 1 and 2 show protection times of the essential oils of candidate plants at 10% and 20% concentrations respectively, as compared to the standard DEET against *An. arabiensis*. All showed complete protection against *An. arabiensis* at 10% and 20% concentrations for freshly applied oils soon after the time of application. At 10% test concentration, only *O. suave* protected biting of laboratory reared *An. arabiensis* for 1 hour with a percentage protection of 83%. The other five oils provided less than an hour of protection and biting rates were similar for both treated and untreated arms at 3 hours. Repellent effect of the standard formulated DEET lasted much longer and provided 83-100% relative protection of up to 6 hours, and declined to 74% and 66% protection after 7 and 8 hours respectively against *An. arabiensis* (Fig.1). Statistical comparison between the six oils with the standard DEET showed that DEET and 10% *O. suave* were not significantly different in efficacy after 1 hour of application. Similarly, after 3 hours of application, all the six oils were not significantly different to each other in efficacy ($p > 0.05$) (Annex 7).

When concentration of the essential oils was increased to 20%, *O. suave* gave the highest protection and the longest duration of protection giving 81.8-100% relative protection of up to 4 hours, but declined to 55% after 5-8 hours of application against *An. arabiensis*. *L. adoensis* at 20% concentration gave 89-100% relative protection for 2 hours against *An. arabiensis*. However, the other oils of the test plants (*E. citriodora*, *E. globulus*, *S. molle* and *O. lamiifolium*) did not provide even 1 hour of complete protection against *An. arabiensis*, and after 3 hours biting rates were similar for both treated and untreated arms. The standard formulated DEET provided 83-100% relative protection for six hours, but declined to 74% and 66% after seven and eight hours of application against *An. arabiensis* (Fig.2). Statistical comparison between DEET, 20% *L. adoensis* and 20% *O. suave* indicated insignificant difference in efficacy ($p > 0.05$) after 2 hours of application. But after 3 and 4 hours of application, DEET and 20% *O. suave* were not significantly different in efficacy ($p > 0.05$) (Annex 8).

Multiple statistical comparisons between 10% and 20% test concentrations of the six oils and DEET indicated that 20% *O. lamiifolium*, 10% *O. suave*, 20% *O. suave*, 20% *L. adoensis* and DEET were not significantly different in efficacy after 1 hour of application against *An. arabiensis* ($p>0.05$). After 2 and 3 hours of application, 20% *L. adoensis*, 20% *O. suave* and DEET were not significantly different in efficacy ($p>0.05$) and after 4 and 5 hours of application, 20% *O. suave* and DEET were not significantly different in efficacy against *An. arabiensis* ($p>0.05$) (Annex 9).

Figures 3 and 4 show protection times of the essential oils of candidate plants at 10% and 20% test concentrations respectively as compared to the standard DEET against *Ae. aegypti*. All essential oils showed complete protection of arms (hands) exposed to adult mosquitoes soon after their application. At 10% concentration, *O. suave* provided 88-100% protection of up to 3 hours against *Ae. aegypti*, but progressively declined from 75% to 30% after 4-8 hours (Fig.3). The next strongest oil, *L. adoensis* gave 80% relative protection for an hour and declined to 62%, 49%, 35%, 24% and 8% protection at 2, 3, 4, 5 and 6 hours, respectively. The remaining four essential oils gave below 60% protection within after an hour and much less than 20% protection after 3 hours of application. The commercial formulated DEET provided 84-100% relative protection after 6 hours and declined to 75% and 67% after 7 and 8 hours of application, respectively. Statistical comparison showed that DEET, 10% *O. suave* and 10% *L. adoensis* were not significantly different in efficacy after 1 hour of application ($p>0.05$). After 2 and 3 hours of application, DEET and 10% *O. suave* were not significantly different in efficacy ($p>0.05$) (Annex 10).

When concentration of the essential oils was increased to 20%, *O. suave* provided 85-100% relative protection up to 5 hours, but declined to 75%, 65% and 56% after 6, 7 and 8 hours respectively against *Ae. aegypti* (Fig.4). The next potential plant oil, *L. adoensis* provided 93-100% relative protection 1 hour post application and declined to 77%, 57%, 46%, 29% and 10% after 2, 3, 4, 5 and 6 hours post application, respectively. None of the rest of the oils provided complete protection for even 1 hour, they provided about 60% protection within an hour and less than 30% within 3 hours of post application against *Ae.*

aegypti. The standard formulated DEET provided 84-100% protection of up to 6 hours and declined to 75% and 67% after 7 and 8 hours, respectively. Statistical comparison showed that DEET, 20% *O. suave* and 20% *L. adoensis* were not significantly different in efficacy after 1 hour of application ($p>0.05$). After 5 hours of application, DEET and 20% *O. suave* were not significantly different in efficacy ($p>0.05$) (Annex 11).

Against *Ae. aegypti*, multiple statistical comparison indicated that in efficacy 10% *L. adoensis*, 20% *L. adoensis*, 10% *O. suave*, 20% *O. suave* and DEET were not significantly different after 1 hour of application ($p>0.05$). After 2 and 3 hours of application 10% *O. suave*, 20% *O. suave* and the standard DEET were not significantly different and 20% *O. suave* and DEET were not significantly different after 4 and 5 hours of application against *Ae. aegypti* ($p>0.05$) (Annex 12).

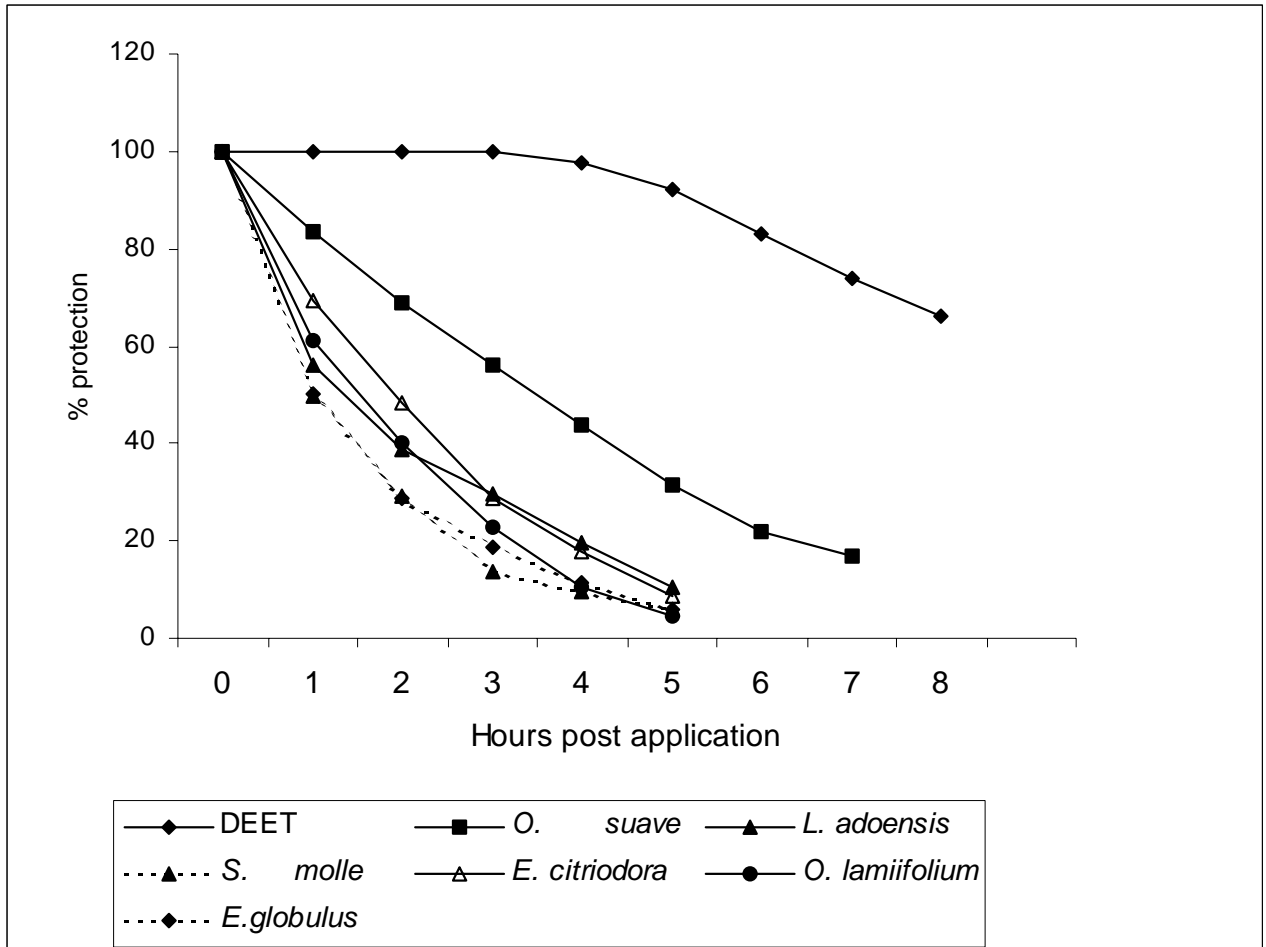


Figure 1 Mean percentage protection versus time (in hour) provided by the essential oils of the test plants and the standard DEET against *Anopheles arabiensis* at 10% concentration.

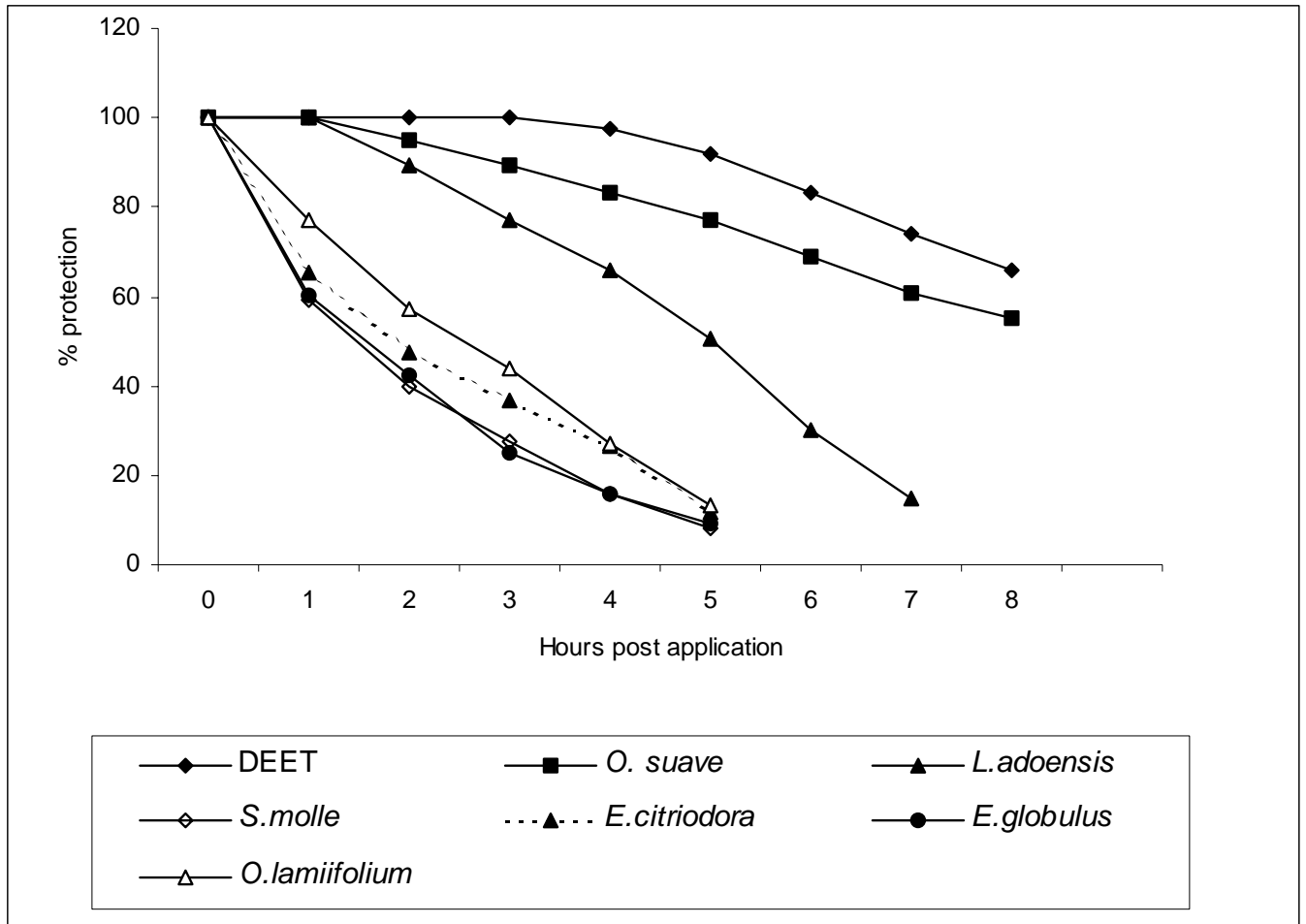


Figure 2 Mean percentage protection versus time (in hour) provided by the essential oils of the test plants and the standard DEET against *Anopheles arabiensis* at 20% concentration.

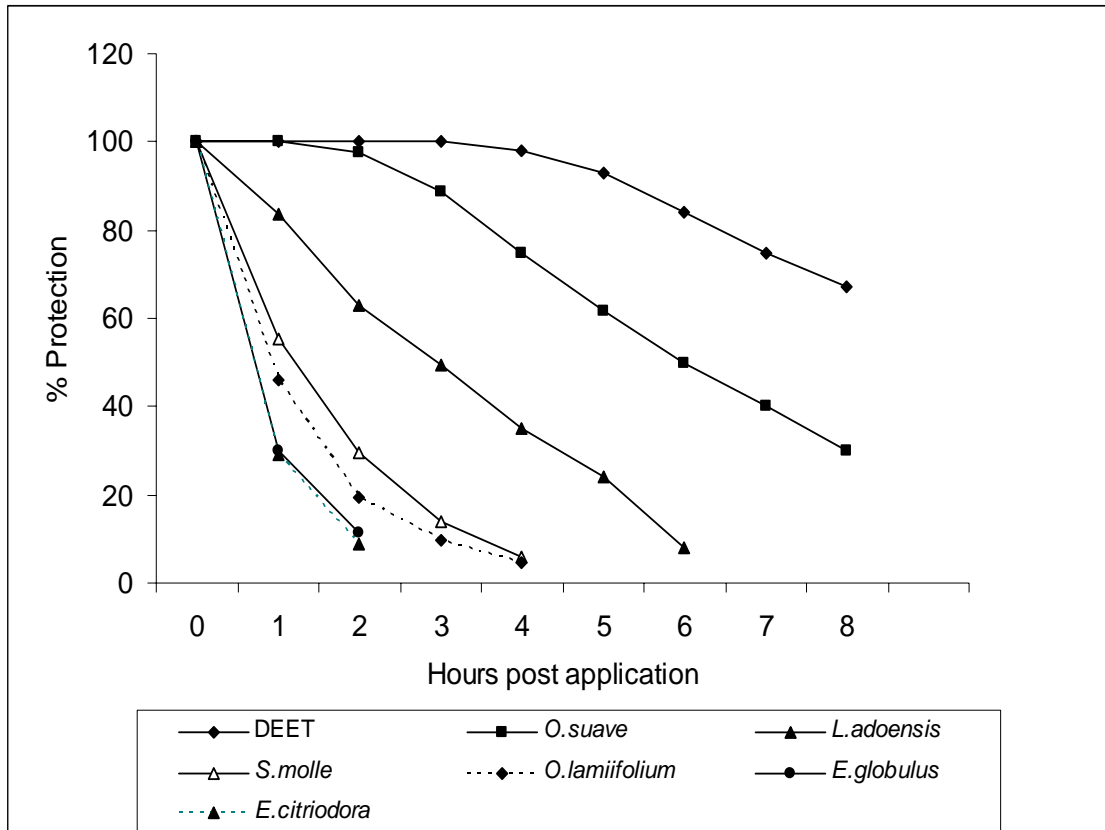


Figure 3 Mean percentage protection versus time (in hour) provided by the essential oils of the test plants and the standard DEET against *Aedes aegypti* at 10% concentration.

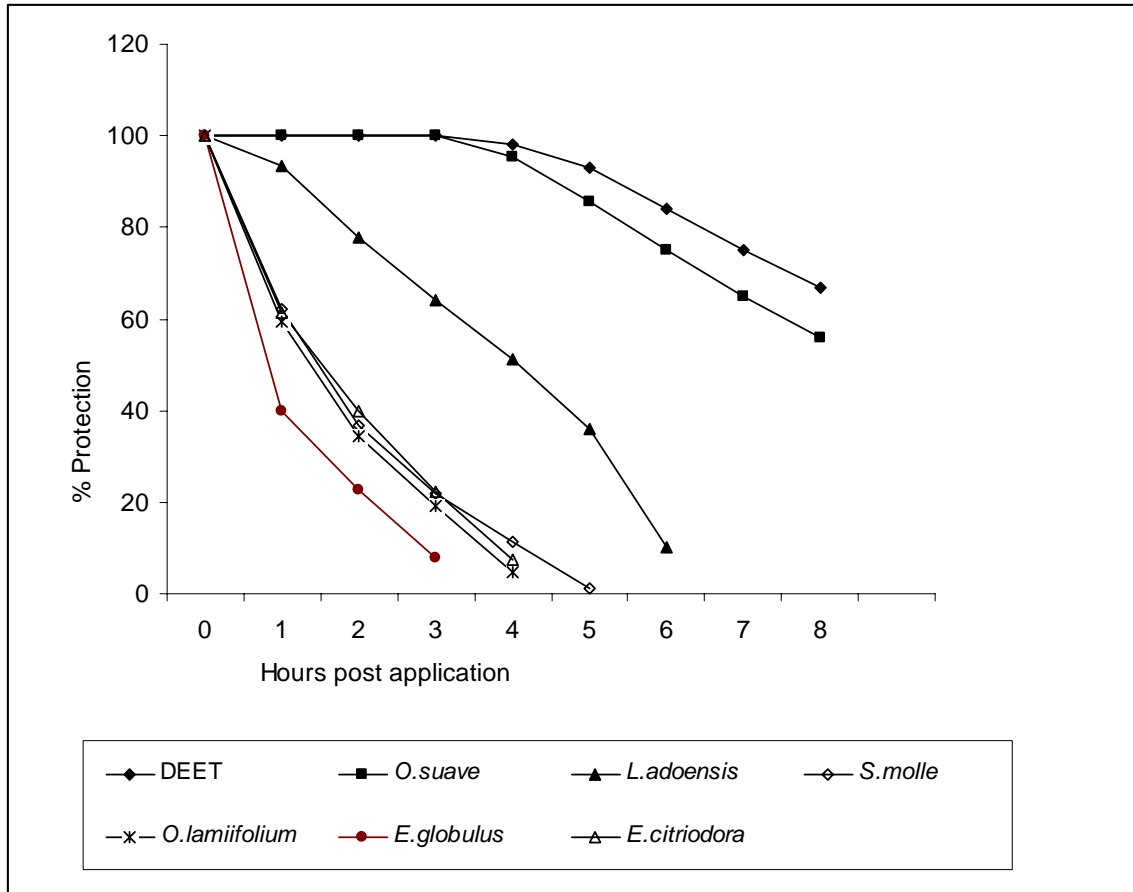


Figure 4 Mean percentage protection versus time (in hour) provided by the essential oils of the test plants and the standard DEET against *Aedes aegypti* at 20% concentration.

4.3 Blend effects of the essential oils

Tables 3 and 4 show the protection times of the blend effects of the 15 combinations of the six essential oils as compared to the standard DEET at 10% and 20% concentrations against *An. arabiensis*. At 10% concentration, all the fifteen combinations showed complete protection soon after application against *An. arabiensis*. *O. suave* + *O. lamiifolium* provided 80-100% protection for 3 hours (the strongest combination) and declined to 35% 4-8 hours after application. *O. suave* combined with the rest of the four oils with 1:1 ratio provided 80-100% protection of up to 2 hours. *O. suave* + *L. adoensis* provided 92-100%, *O. suave* + *S. molle* provided 83-100%, *O. suave* + *E. citriodora* provided 73% and *O. suave* + *E. globulus* provided 78% protection of up to 2 hours and declined to 28%, 27%, 15% and 22% after 3 to 8 hours post application respectively. The other ten combinations: *L. adoensis* + *S. molle*, *L. adoensis* + *E. citriodora*, *L. adoensis* + *E. globulus*, *L. adoensis* + *O. lamiifolium*, *E. citriodora* + *E. globulus*, *E. citriodora* + *S. molle*, *E. citriodora* + *O. lamiifolium*, *E. globulus* + *S. molle*, *E. globulus* + *O. lamiifolium* and *S. molle* + *O. lamiifolium* provided less than 80% protection after an hour of application. But the standard formulated DEET provided much longer 83-100% protection for 6 hours and declined to 66% after 8 hours of application respectively (Table 3).

When concentration of the combined oils increased to 20%, *O. suave* + *O. lamiifolium* provided 81-100% protection for 4 hours, *O. suave* + *L. adoensis* provided 89-100% for 3 hours, *O. suave* + *S. molle* provided 92-100% protection for 3 hours *O. suave* + *E. globulus* provided 97-100% protection for 3 hours and *O. suave* + *E. citriodora* provided 86-100% protection for 3 hours after application and declined to 39% 5-8 hours, 40%, 36%, 36% and 36% after 4-8 hours of application, respectively against *An. arabiensis*. Out of the other ten combinations at 20% concentrations, *E. globulus* + *O. lamiifolium* provided 80-100% protection for 2 hours, *S. molle* + *E. globulus* provided 85-100% protection for 1 hour, *S. molle* + *O. lamiifolium*, *E. citriodora* + *O. lamiifolium* and *L. adoensis* + *E. globulus* provided 80-100% protection for 1 hour after application against *An. arabiensis*. The other five combinations provided less than 80% protection after an

hour of application against *An. arabiensis*. The standard formulated DEET provided 83-100% protection for 6 hours after application and declined to 74% and 66% after 7 and 8 hours of application (Table 4).

Statistical comparison of 10% concentration of the fifteen combinations against *An. arabiensis* indicated that *O. suave* + *E. citriodora*, *O. suave* + *E. globulus*, *O. suave* + *L. adoensis*, *O. suave* + *O. lamiifolium*, *O. suave* + *S. molle* and the standard DEET were not significantly different in efficacy after 1 hour of application ($p>0.05$). After 2 hours of application *O. suave* + *O. lamiifolium*, *O. suave* + *L. adoensis* and DEET were not significantly different in efficacy ($p>0.05$) (Annex 13). But at 20% concentration, *E. globulus* + *S. molle*, *E. globulus* + *O. lamiifolium*, *O. suave* + *L. adoensis*, *O. suave* + *E. citriodora*, *O. suave* + *E. globulus*, *O. suave* + *S. molle*, *O. suave* + *O. lamiifolium* and DEET were not significantly different after 1 hour of application in efficacy ($p>0.05$). *O. suave* + *E. citriodora*, *O. suave* + *L. adoensis*, *O. suave* + *E. globulus*, *O. suave* + *S. molle*, *O. suave* + *O. lamiifolium* and DEET were not significantly different in efficacy after 2 and 3 hours of application ($p>0.05$) (Annex 14).

Multiple statistical comparison between 10% and 20% concentrations of the fifteen combinations against *An. arabiensis* showed that 10% *O. suave* + *E. globulus*, 10% *O. suave* + *L. adoensis*, 10% *O. suave* + *O. lamiifolium*, 10% *O. suave* + *S. molle*, 20% *E. globulus* + *S. molle*, 20% *E. globulus* + *O. lamiifolium*, 20% *O. suave* + *L. adoensis*, 20% *O. suave* + *E. citriodora*, 20% *O. suave* + *E. globulus*, *O. suave* + *S. molle*, 20% *O. suave* + *O. lamiifolium* and DEET were not significantly different in efficacy after 1 hour of application ($p>0.05$). After 2 hours of application, 10% *O. suave* + *O. lamiifolium*, 10% *O. suave* + *L. adoensis*, 20% *O. suave* + *E. citriodora*, 20% *O. suave* + *L. adoensis*, 20% *O. suave* + *E. globulus*, 20% *O. suave* + *S. molle*, 20% *O. suave* + *O. lamiifolium* and DEET were not significantly different in efficacy ($p>0.05$). But after 3 hours of application only 20% *O. suave* + *E. citriodora*, 20% *O. suave* + *L. adoensis*, 20% *O. suave* + *E. globulus*, 20% *O. suave* + *S. mole*, 20% *O. suave* + *O. lamiifolium* and DEET were not significantly different in efficacy ($p>0.05$) (Annex 15).

Table 3. Mean percentage protection \pm SE of 1:1 combination of 10% concentration of essential oils against *An. arabiensis* at different times after application of oils

Combined oils	Hours post application							
	1	2	3	4	5	6	7	8
<i>Os + Ol</i>	95.0 \pm 5	88.0 \pm 4.6	80.0 \pm 2.8	70.0 \pm 2.8	59.0 \pm 2.3	50 \pm 2.1	42 \pm 2.4	35 \pm 3.1
<i>Os + Sm</i>	100.0 \pm 0	83.0 \pm 1.7	74.0 \pm 2.3	61.0 \pm 0.5	52.0 \pm 1.1	43 \pm 1.1	34 \pm 2.2	27 \pm 0.5
<i>Os + La</i>	94.0 \pm 0.5	92.0 \pm 1.1	75.0 \pm 2.8	59.0 \pm 2.3	49.0 \pm 0.5	40 \pm 1.6	34 \pm 2.8	28 \pm 1.1
<i>Os + Eg</i>	92.0 \pm 0.5	78.0 \pm 2.3	65.0 \pm 1.1	53.0 \pm 0.5	42.0 \pm 0.5	35 \pm 0.5	28 \pm 3.4	22 \pm 0.5
<i>Os + Ec</i>	90.0 \pm 1.7	73.0 \pm 1.1	55.0 \pm 2.8	42.0 \pm 1.1	32.0 \pm 0.5	24 \pm 2.3	19 \pm 1.1	15 \pm 0.5
<i>La + Ec</i>	63.0 \pm 1.7	38.0 \pm 1.1	25.0 \pm 2.8	16.0 \pm 3.4	3.3 \pm 2.4	-	-	-
<i>La + Eg</i>	72.0 \pm 1.1	50.0 \pm 2.8	32.0 \pm 0.5	21.0 \pm 0.5	7.0 \pm 1.1	-	-	-
<i>La + Sm</i>	64.0 \pm 2.3	45.0 \pm 2.8	30.0 \pm 1.1	19.0 \pm 0.5	10.0 \pm 0.5	-	-	-
<i>La + Ol</i>	64.0 \pm 1.1	45.0 \pm 1.1	31.0 \pm 0.5	18.0 \pm 0.5	8.0 \pm 0	-	-	-
<i>Ec + Eg</i>	66.0 \pm 1.5	41.0 \pm 1.7	27.0 \pm 0	15.0 \pm 2.8	5.0 \pm 2.8	-	-	-
<i>Ec + Sm</i>	68.0 \pm 1.1	45.0 \pm 2.8	29.0 \pm 0.5	21.0 \pm 0.5	10.0 \pm 2.8	-	-	-
<i>Ec + Ol</i>	65.0 \pm 2.8	51.0 \pm 1.7	37.0 \pm 1.7	25.0 \pm 2.8	15.0 \pm 2.8	-	-	-
<i>Eg + Sm</i>	76.0 \pm 3.4	59.0 \pm 1.1	43.0 \pm 1.7	30.0 \pm 2.8	15.0 \pm 0	-	-	-
<i>Eg + Ol</i>	64.0 \pm 2.3	46.0 \pm 3.4	32.0 \pm 1.1	20.0 \pm 1.1	12.0 \pm 0.5	-	-	-
<i>Sm + Ol</i>	79.0 \pm 0.5	60.0 \pm 1.1	41.0 \pm 0.5	29.0 \pm 0.5	12.0 \pm 1.1	-	-	-
DEET	100 \pm 0	100 \pm 0	100 \pm 0	97.6 \pm 1.2	92.0 \pm 2.5	83 \pm 2.2	74 \pm 1.4	66 \pm 2.6

Key: 1. *Os* = *O. suave* 4. *Ec* = *E. citriodora*
2. *La* = *L. adoensis* 5. *Eg* = *E. globules*
3. *Sm* = *S. molle* 6. *Ol* = *O. lamiifolium*

Table 4. Mean percentage protection \pm SE of 1:1 combination of 20% concentration of essential oils against *An. arabiensis* at different times after application of oils

Combined oils	Hours post application							
	1	2	3	4	5	6	7	8
<i>Os + Ol</i>	100 \pm 0	100 \pm 0	91.6 \pm 1.6	81 \pm 2.3	69.3 \pm 0.8	57 \pm 2.4	47 \pm 3.7	39 \pm 3.4
<i>Os + Sm</i>	100 \pm 0	97.6 \pm 2.3	91.6 \pm 3.3	79.3 \pm 3.2	66.3 \pm 2.7	55 \pm 1.7	44 \pm 5	36 \pm 2.3
<i>Os + La</i>	100 \pm 0	97.6 \pm 2.3	88.6 \pm 4.3	77.3 \pm 5.2	66.6 \pm 5.9	57 \pm 3.3	48 \pm 0.5	40 \pm 1.8
<i>Os + Eg</i>	100 \pm 0	97.6 \pm 2.3	87.6 \pm 4.4	76 \pm 4.5	67 \pm 4	54 \pm 0.8	45 \pm 1.5	36 \pm 2.7
<i>Os + Ec</i>	100 \pm 0	96 \pm 2	85.6 \pm 3.1	72 \pm 4	59 \pm 3.5	49 \pm 2.9	42 \pm 3.5	36 \pm 4.2
<i>La + Ec</i>	64 \pm 4	40.6 \pm 4.3	26.6 \pm 3.1	16.6 \pm 4.4	10.3 \pm 5.2	-	-	-
<i>La + Eg</i>	80.6 \pm 4.7	62.6 \pm 1.7	47.6 \pm 1.2	36.6 \pm 0.8	30 \pm 0.5	-	-	-
<i>La + Sm</i>	79 \pm 3.4	64.6 \pm 3.2	54.3 \pm 3.2	44 \pm 4	35.6 \pm 2	26 \pm 3.4	12 \pm 1.7	-
<i>La + Ol</i>	78.6 \pm 7	61 \pm 2.3	43 \pm 3.2	30.3 \pm 2.9	12.3 \pm 6.2	-	-	-
<i>Ec + Eg</i>	77 \pm 13	51.3 \pm 7.9	32 \pm 10	23 \pm 5.1	15.6 \pm 4.7	-	-	-
<i>Ec + Sm</i>	76.3 \pm 8	61.3 \pm 4.4	49.3 \pm 2.9	37.3 \pm 2.7	28 \pm 2.3	-	-	-
<i>Ec + Ol</i>	80.3 \pm 9.4	67.6 \pm 2.9	54 \pm 3	40.6 \pm 2.3	28.3 \pm 2.7	-	-	-
<i>Eg + Sm</i>	85.3 \pm 3.6	65 \pm 2	46 \pm 1	29.6 \pm 2	21.3 \pm 1.7	-	-	-
<i>Eg + Ol</i>	94.3 \pm 2.8	80.3 \pm 1.4	64.3 \pm 1.7	47.6 \pm 1.2	35.6 \pm 0.8	25 \pm 2.2	-	-
<i>Sm + Ol</i>	80.3 \pm 2.4	64.6 \pm 5.5	51 \pm 1.7	38 \pm 2.8	28 \pm 2.3	-	-	-
DEET	100 \pm 0	100 \pm 0	100 \pm 0	97.6 \pm 1.2	92 \pm 2.5	83 \pm 1.9	74 \pm 3	66 \pm 4.1

Key: 1. *Os* = *O. suave*

2. *La* = *L. adoensis*

3. *Sm* = *S. molle*

4. *Ec* = *E. citriodora*

5. *Eg* = *E. globules*

6. *Ol* = *O. lamiifolium*

5 DISCUSSION

The present study was conducted to evaluate repellency effect of essential oils of six plants against laboratory colonies of *An. arabiensis* and *Ae. aegypti*. 10% and 20% concentrations were used to compare the efficacy of the oils. At 10% concentration essential oil of *O. suave* has shown 83.3% repellency after an hour of application and the other five oil provided less than 70% repellency against *An. arabiensis*. At 20% concentration *O. suave* provided 83% repellency after 4 hours of application, *L. adoensis* provided 89.6% repellency after 2 hours and the other four oils provided less than 80% repellency against *An. arabiensis*. At 10% concentration *O. suave* provided 88.6% repellency after 3 hours of application, *L. adoensis* provided 83.6% repellency after 1 hour of application and the other four oils provided less than 50% repellency after 1 hour of application against *Ae. aegypti*. At 20% concentration *O. suave* provided 85.6% repellency after 5 hours of application, *L. adoensis* provided 93.6% repellency after 1 hour of application and the other four oils provided less than 65% repellency against *Ae. aegypti*.

In the blend effect study, 1:1 combinations of *O. suave* with the five oils showed strong repellent activity against *An. arabiensis* at 10% and 20% test concentrations. At 10% concentration *O. suave* + *O. lamiifolium* provided 80% repellency after 3 hours of application, *O. suave* + *L. adoensis* and *O. suave* + *S. molle* provided 92% and 83% repellency after 2 hours of application respectively and *O. suave* + *E. globulus* and *O. suave* + *E. citriodora* provided 92% and 90% repellency after 1 hour of application against *An. arabiensis* respectively. At 20% concentration *O. suave* + *O. lamiifolium* and *O. suave* + *S. molle* provided 81% and 79.3% repellency after 4 hours of application respectively, and *O. suave* + *L. adoensis*, *O. suave* + *E. globulus* and *O. suave* + *E. citriodora* provided 88.6%, 87.6% and 85.6% repellency after 3 hours of application respectively against *Ae. aegypti*. Another combination, *E. globulus* + *O. lamiifolium* provided 83.3% repellency after 2 hours of application at 20% concentration.

The essential oils from all the test plants were effective both at 10% and 20% concentration against *An. arabiensis* and *Ae. aegypti* and 1:1 combinations of the six oils against *An. arabiensis*. Although essential oils of all the test plants and 1:1 combinations of the oils were repellent, duration of complete protection was less than an hour for four essential oils (*E. citriodora*, *E. globulus*, *S. molle* and *O. lamiifolium*) and 1:1 combinations of the five oils (*L. adoensis*, *S. molle*, *O. lamiifolium*, *E. citriodora* and *E. globulus*) both at 10% and 20% concentrations. The low duration of protection suggests that there is faster loss of repellent effect due to faster volatilization of compounds of the essential oils rather than lack of effectiveness. To extend the protection time, appropriate formulations may be used.

The protection time of essential oils from plants turmeric (*Curcuma longa*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*O. americanum*) was significantly increased by addition of 5% vanillin against three mosquito vectors (*Ae. aegypti*, *An. dirus* and *Culex quinquefasciatus*) in a study conducted in Thailand (Tawatsin *et al.*, 2001). The protection time of *E. globulus* was prolonged from 3-5 hours after adding 5% vanillin against *Ae. albopictus* under laboratory condition in china (Yang and Ma, 2005). The use of controlled release formulations with topical repellents (disintegration of the carrier agent releases repellent vapor slowly to prevent bites) has provided prolonged protection against biting mosquitoes in the laboratory under three climatic regimes of environmental chambers (variable humidity, constant high humidity and hot condition) (Gupta and Rutledge, 1989).

The duration of protection exhibited by the essential oils in the present study (except *O. suave*) is comparable to the ones reported by previous workers for citronella oil against *An. gambiae* (Trigg and Hill, 1996). It can also be compared to *O. suave*, *O. americanum*, *O. kilimandscharicum* and *Lantana camara* against *An. gambiae* which have shown less than an hour complete protection in laboratory studies in Kenya (Seyoum, 2003). Citronella oil has long been used in commercial preparations of repellents and is popular in India, though generally rated as less effective than repellents with synthetic active ingredients (Curtis *et al.*, 1987).

Repellency of essential oils of *O. suave* against *Ae. aegypti* was earlier reported with results similar to the present study showing mean protection time of over three hours (Chogo and Crank, 1981); against *Rhipicephalus appendiculatus* tick, as it repel and kill the active stages (Mwangi *et al.*, 1995). In east Africa, *O. suave* oil did not provide complete protection even an hour at 20% concentration against *An. gambiae s.s.* (Seyoum, 2003). But, in the present study (which is the first in Ethiopia) it provided 82-100% relative protection for four hours against *An. arabiensis* and 85-100% relative protection for five hours against *Ae. aegypti* at 20% concentration. This shows wide variation in the sensitivity of different species of insects to repellent oils. Rutledge *et al.* (1978) reported that differences in the sensitivity to a given repellent (DEET) were a function of the taxonomic distance between the anopheline and culicine mosquitoes and individual mosquitoes within the strains tested differed in their level of sensitivity. Accordingly a repellent test data obtained from one species of mosquito can not be used to predict its effectiveness against another species.

The evaluation of essential oil of *O. lamiifolium* for its repellency against mosquitoes is the first in Ethiopia and elsewhere. However, it provided less than an hour protection against *An. arabiensis* and *Ae. aegypti*. Atsebeha (2005) reported that only fresh leaves of *O. lamiifolium* showed effective repellency to *An. pharoensis* and a non-repellency effect to *An. arabiensis* and culicines in field studies. In several studies, other *Ocimum* species have been tested for efficacy in repelling insects. Fresh *O. canum* is occasionally used in Guinea Bissau as mosquito repellent and was found that it had strong mosquito repellent property providing 63.6% by smouldering in the field (Palsson and Jaenson, 1999). But thermal expulsion and periodic direct burning of the leaves of *O. amaricanum* in a semi field experimental hut in two villages in western Kenya provided satisfactory results against *An. gambiae s.l.* and *An. funestus* (Seyoum *et al.*, 2002a). In Kenya, essential oils of *O. forskolei* and *O. fischeri* were evaluated for repellency on forearms of human volunteers against *An. gambiae s.s.* and were found to be more repellent than DEET (Odalo *et al.*, 2005). *O. selloi* oil has been found to be an effective repellent at 10% concentration providing 89% repellency for 1 hour when the highest prevalence of

mosquitoes was noted in the field condition in Brazil against *An. braziliensis* (Padilha de Paula *et al.*, 2003). The protection times and percentages of repellency for 20% *O. basilicum* oil were 120 minutes and 81.1%, 210 minutes and 66.7% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus*, respectively in laboratory studies (Amer and Mehlhorn, 2006). Differences in plant species and test mosquitoes may be the reasons for variation of repellent effects of the oils (Rutledge *et al.*, 1978; Rutledge, 1983; Robert *et al.*, 1991).

The essential oils of *E. globulus* and *E. citriodora* showed similar repellency effects against *An. arabiensis* and *Ae. aegypti* in the present study. They provided less than an hour complete protection at both 10% and 20% concentrations. Similarly, *E. globulus* provided less repellency against *An. arabiensis*, *An. pharoensis* and culicines in direct burning and thermal expulsion methods (Atsebeha, 2005). In other experiments with essential oil of *E. globulus* in the laboratory, Yang and Ma (2005) reported that 15% concentration of the oil gave protection to humans for at least three hours against *Ae. albopictus*. Choi *et al.* (2002) reported that the oil was found to be effective repellent against adult mosquitoes of *Culex pipiens pallens* on hairless mice with a protection rate of 70% and at a concentration of 0.5% topical treatment. Amer and Mehlhorn (2006) reported that protection times and percentages of repellency for 20% *E. globulus* oil were 60 minutes and 57.6%, 330 minutes and 52.4% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus*, respectively. 20% *E. citriodora* oil in the same experiment gave protection times and percentages of repellency of 150 minutes and 59.4%, 480 minutes and 52.4% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus*, respectively (Amer and Mehlhorn, 2006). Many factors such as different mosquito densities in the cages and cage size (Barnard *et al.*, 1998) and different test mosquito species with different sensitivity to repellent oils (Rutledge *et al.*, 1978; Rutledge, 1983; Robert *et al.*, 1991) may contribute for these variations in the repellent activity of the oils.

There were good reports from other countries about the repellency of *Eucalyptus* species against vector mosquitoes. The leaves of *Eucalyptus* species are used both outdoors and indoors as mosquito repellent in Guinea Bissau (Palsson and Jaenson, 1999). The protection times and percentages of repellency for narrow-leaved (*E. radiata*) and broad-leaved (*E. dives*) eucalyptus oils gave over three to eight hours and over 42-100% protection against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus* (Amer and Mehlhorn, 2006). Comparison of a repellent based on the lemon Eucalyptus (*E. citriodora*), containing as the main active component *p*-menthane-3,8-diol against *Ae. albopictus* and *An. quadriannulatus* applied as >2× DEET on the skin of human volunteers showed this repellent to be as effective as DEET (Schreck and Leonhardt, 1991). Comparison of lemon Eucalyptus based repellent with two commercial repellents containing 20-22% DEET against *An. gambiae* showed that the three repellents did not differ significantly (Collins and Brady, 1993).

In Tanzania in a similar manner, a lemon Eucalyptus based repellent was evaluated in the field in comparison with DEET against *An. gambiae* and *An. funestus* and gave complete protection from biting for between 6 and 7 hours, depending up on the formulation type, with significant differences between the Eucalyptus based repellent and DEET in terms of efficacy and duration of protection (Trigg, 1996). MosiguardTM, a repellent containing *p*-menthane-3,8-diol was tested against *An. arabiensis* in comparison with DEET in the laboratory in South Africa and was found to be comparable to DEET giving 90-100% relative protection up to 5-6 hours (Govere *et al.*, 2000). However, in the present study, DEET provided much longer duration of protection than 20% concentration of *E. citriodora* oil and the difference between the efficacy of the essential oil of *E. citriodora* and previous studies was that Eucalyptus based repellent contains the principal active ingredient *p*-menthane-3,8-diol (quwenling) that is obtained from the waste distillate after extraction of essential oil from lemon Eucalyptus plant (Schreck and Leonhardt, 1991).

In the present study, the essential oils of *L. adoensis* showed 89-100% protection for two hours at 20% concentration against *An. arabiensis* and 93-100% protection for an hour against *Ae. aegypti*. Similar experiments made on other *Lippia* species exhibited

relatively high repellency. Protection times and percentages of repellency for 20% *L. citriodora* provided 150 minutes and 70.3%, 330 minutes and 38.1% and 480 minutes and 100% against *Ae. aegypti*, *An. stephensi* and *Culex quinquefasciatus* respectively in the laboratory studies (Amer and Mehlhorn, 2006). The oils of *L. javanica* and *L. ukambensis* exhibited high repellency (the authors did not give percentage repellency), comparable to the synthetic repellent DEET against *An. gambiae* s.s. in Kenya (Omolo *et al.*, 2004). The repellency largely accounted for respective constituents present in the essential oils of *L. ukambensis* and *L. javanica*. In *L. ukambensis*, the constituents camphor, camphene, 1,8-cineole are in ratio 40:9:2.5 and in *L. javanica*, the constituents limonene oxide, *cis*-verbenol, verbenone, linanool, limonene, α -terpeneol are in ratio 39:11: 6:3:2.5:2. The constituents in *L. adoensis* in Ethiopia as determined by Asres and Bucar (2002) are *E*-Citral (=Geraniol), *Z*-Citral (=Neral), Limonene, Carvone, Germacrene D, *trans*-Geraniol, Nerol, Camphor, Cineol, Pipritenone and *trans*- β -Ocimene are in ratio 16.9: 13: 11.8: 10.4: 6.2: 3.4: 2.6: 2.3: 2.1: 2.1: 2. In Zimbabwe, Lukwa *et al.* (1999) reported that 50% of the study subjects use *L. javanica* as mosquito repellent by smoking and placing fresh plant parts.

At 20% and 10% concentrations, the essential oil of *S. molle* is less effective against *An. arabiensis* and *Ae. aegypti*. Atsebeha (2005) reported that direct burning and periodic thermal expulsion of the seeds of *S. molle* exhibited profound repellency effect on *An. arabiensis* and *An. pharoensis*, but the leaves provided good repellency against only *An. arabiensis*. However, the essential oil of *S. molle* has not been evaluated against mosquitoes and other biting insects as a repellent in Ethiopia and elsewhere.

Regarding blend effects, evaluation of *O. suave* + *O. lamiifolium* at 10% concentration gave the maximum protection time of up to 3 hours with 80%-100% repellency. When the same combination was increased to 20% concentration, the protection time was increased to 4 hours with 81%-100% repellency. When *O. suave* was combined with *S. molle* at 10% concentration, 2 hours protection was afforded with 83%-100% repellency. When the concentration of this mixture was increased to 20%, the protection time was also increased to 4 hours with about 80%-100% repellency. When *O. suave* was mixed

with *L. adoensis* at 10% concentration, protection time was only for 2 hours with about 92%-100% repellency. Increasing the concentration of this mixture to 20%, protection was also increased to 3 hours with about 88%-100% repellency. Similarly, a mixture of *O. suave* and *E. globulus* at 10% concentration gave only 1 hour of protection with about 92%-100% repellency. The 20% concentration of the same mixture however gave 3 hours of protection with about 87%-100% repellency. When *O. suave* was mixed with *E. citriodora* at 10% concentration, it gave only 1 hour protection with 90%-100% repellency. At 20% concentration, the same mixture gave increased protection for about 3 hours with 85%-100% repellency. Other mixtures gave less protection time with less than 80% repellency, although the same trend of increased repellency was observed when the concentration was increased to 20%.

Studying the repellency of essential oils to mosquitoes Barnard (1999) combined 50% concentration of the essential oil of clove with 50% concentration of essential oil of geranium or with thyme oil (50%) that prevented biting by *An. albimanus* for 1¼ to 2½ hours. Moreover, repellency of the oil mixtures to *An. albimanus* exceeded that of DEET and was greater than the sum of the complete protection times for their constituent oils in each case, except 25% clove oil + 75% geranium oil mixture, but none of the oil combinations repelled *Ae. aegypti*. Amer and Mehlhorn (2006) also evaluated complex mixtures of oils at lower concentrations (1% or 2% of each) in different solvents and formulations from five best oils (*Litsia cubeba*, *Melaleuca leucandron*, *Melaleuca quinquenervia*, *Viola odorata*, *Nepeta cataria*) at 20% concentration. Out of eleven mixtures tested, five mixtures were excellent repellent products against *Ae. aegypti* mosquitoes offering 480 minutes of protection, however their percentages of repellency ranged from 70.3%-100% in the laboratory studies.

It can be therefore suggested that combination of essential oils can increase the repellent activity to some species of mosquitoes. In the present study as well, it can be seen that repellency could be improved when mixtures of oils are used rather than with single oil application at both 10% and 20% concentration showing synergistic effects of the

concentration of the essential oils. Environmental Protection Agency (EPA) of USA cited in Novak and Gerberg (2005) has set a 2 hour minimum repellent activity of a candidate plant oil in order to gain registration. Based on the present laboratory observation against *An. arabiensis* and *Ae. aegypti*, some plants seem potential candidates as repellents. However, their performance in field situation must be evaluated before being recommended for registration and use. Novak and Gerberg (2005) and EPA strongly recommended that only field studies should be used to establish efficacy and registration. Cage studies should only be used for screening purposes (Novak and Gerberg, 2005).

DEET provided 83-100% and 84-100% relative protection up to six hours against *An. arabiensis* and *Ae. aegypti* and declined to 66% and 67% up to 8 hours respectively in the present study. These results can be compared to the previous studies on DEET against different mosquitoes. DEET, which was first developed by the United States Army in 1946 and registered in 1957 (Fradin, 1998) is a broad spectrum repellent against mosquitoes, biting flies, fleas and ticks (Davis, 1985), marketed in 5-100% concentrations of various formulations. Frances *et al.* (1998) reported that DEET provided 95% protection against *An. faruati* for 5 hours and declined 8 hours after application and provided <50% protection at 9 hours in the field in Australia. Trigg (1996) reported that DEET provided greater than 6 hours protection from biting of *Anopheles* species in Tanzania in the field. In the laboratory in South Africa DEET provided 90-100% protection up to 5-6 hours against *An. arabiensis* (Govere *et al.*, 2000).

6 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Malaria is the most important of the vector borne diseases both in terms of its geographical distribution, incidence and the extent of the morbidity and mortality it causes in Ethiopia. In this country, *An. arabiensis* is the principal vector of malaria followed by *An. pharoensis*, *An. funestus* and *An. nili*. Due to increased insecticide resistance by vector mosquitoes, anti-malarial drug resistance by malaria parasites and environmental changes, the problems caused by malaria is increasing and forcing people to search for alternative control methods. One of these is a search for plant-derived mosquito-repellents, especially essential oils from local medicinal plants being conducted in different countries with no investigations in Ethiopia.

The present study evaluates the essential oils of certain local plants for their repellency against *An. arabiensis* as it is the major vector in Ethiopia and *Ae. aegypti* in the laboratory. Comparison of protection time and percentage repellency were carried out. Of the six essential oils *O.suave* showed the highest repellency effect against *An. arabiensis* and *Ae. aegypti* at 20% concentration followed by *L .adoensis* and the other four oils provided the least repellency. The blend effects of the six oils were studied and the five blends (*O. suave* with the other five oils) also provided remarkable repellency against *An. arabiensis* at 20% concentration. The other ten blends showed less repellency against *An. arabiensis* both at 10% and 20% concentration.

6.2 Recommendation

Even though the appropriate criteria for synthetic repellent products are at least 80% reduction in biting for 6-8 hours after application (Barnard, 2000), the Environmental Protection Agency (EPA) of USA set 2 hours minimum activity requirement of a plant-derived repellent in order to gain registration (Novak and Gerberg, 2005). The essential

oil of *O. suave* mixed with the other five oils, and *L. adoensis* especially at 20% concentration provided satisfactory repellent effect. But selection of a potential plant repellent for further development should not be based on a limited number of test insects because responses to repellents vary within and among species (Rutledge *et al.*, 1978: Rutledge, 1983: Robert *et al.*, 1991). Therefore further studies should be conducted against many different biting arthropods as possible under laboratory and field conditions.

Despite the efficacy of DEET and other synthetic repellents, there are some concerns about their safety. Therefore, the present study of the essential oils must also be evaluated for their safety on the skin. During and after the present investigation no adverse effects on the skin or other parts of the body of human volunteers were observed. Plant-based products are generally regarded as safe compared to synthetic repellents (Novak and Gerberg, 2005).

Methods enhancing the efficacy of repellents such as purification of the active ingredients, reduction of skin absorption, slow release of the repellents and increase in the duration of repellency need to be studied.

7 REFERENCES

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Annex 1. Mean percentage protection of the essential oils at 10% concentration of the test plants against *An. arabiensis*.

Hours post application	% protection (Mean±S.E)						
	<i>O. suave</i>	<i>L. adoensis</i>	<i>S. molle</i>	<i>E. citriodora</i>	<i>E. globulus</i>	<i>O. lamiifolium</i>	DEET
1	83.33±0.88	56.33±9.7	49.66±3.7	69.33±6.7	50.00±12.5	61.00±2.0	100.00
2	69.00±2.0	38.66±8.0	29.33±3.3	48.33±9.2	28.66±10.4	40.00±3.6	100.00
3	56.33±3.2	29.66±4.6	13.66±4.6	28.66±6.4	18.66±6.9	23.00±1.5	100.00
4	44.00±6.0	19.66±2.1	9.66±2.9	18.00±6.2	11.33±4.6	10.33±0.8	97.66±1.2
5	31.33±6.2	10.66±2.0	6.00±2.0	8.66±3.7	6.00±2.6	4.66±0.8	92.00±2.5
6	22.00±4.6	-	-	-	-	-	83.00±2.2
7	17.00±5.3	-	-	-	-	-	74.00±3.0
8	-	-	-	-	-	-	66.00±2.8

Annex 2. Mean percentage protection of the essential oils at 20% concentration of the test plants against *An. arabiensis*.

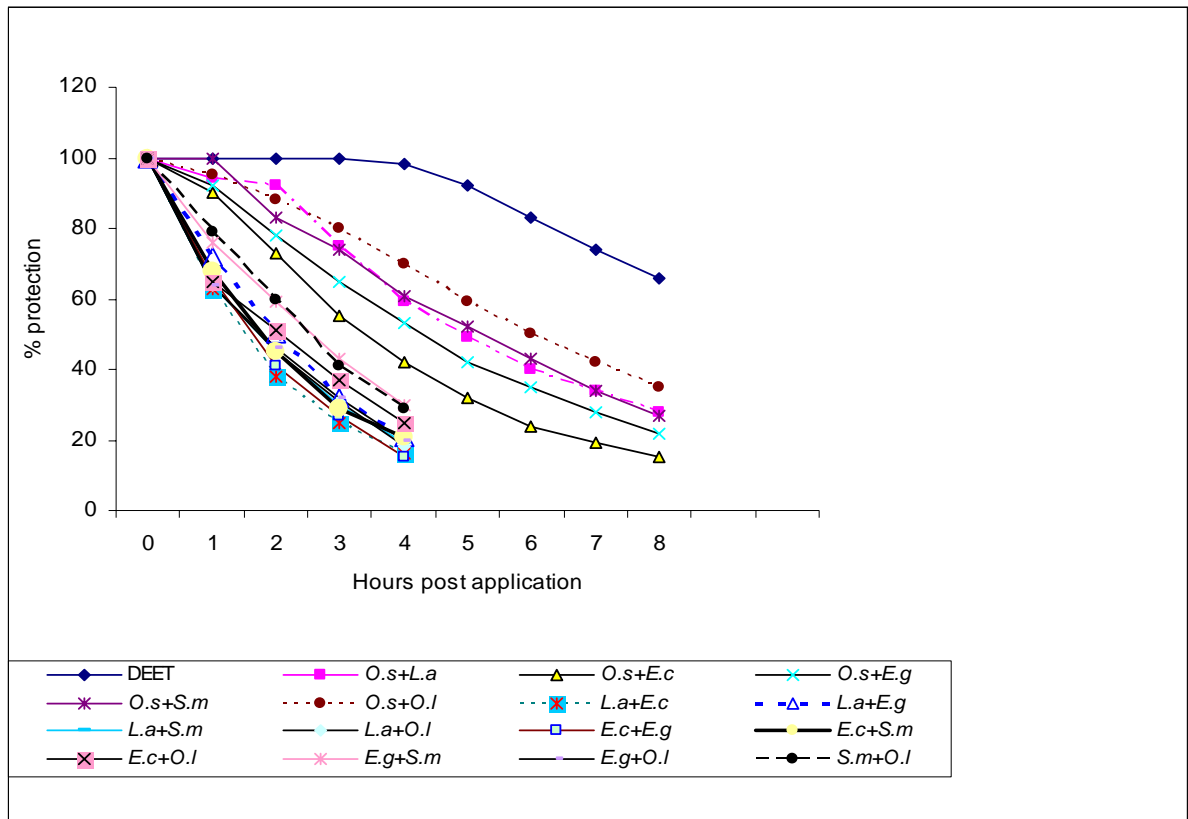
Hours post application	% protection (Mean ± S.E)						
	<i>O. suave</i>	<i>L. adoensis</i>	<i>S. molle</i>	<i>E. citriodora</i>	<i>E. globulus</i>	<i>O. lamiifolium</i>	DEET
1	100.00	100.00	59.0±4.9	65.66±2.6	60.33±5.2	77.00±3.4	100.00
2	95.00±0	89.66±2.3	40.0±4	47.66±3.8	42.66±8.7	57.00±1.7	100.00
3	89.3±1.3	77.33±2.9	27.6±2.7	36.66±1.8	25.00±11	44.00±1.1	100.00
4	83.0±2.3	66.00±2	16.00±2	26.33±0.3	15.66±7.1	27.33±1.4	97.66±1.2
5	77.0±2.6	50.33±5.5	8.33±0.8	11.66±0.8	9.00±5.5	13.33±1.6	92.00±2.5
6	69.0±4.2	30.00±2.3	-	-	-	-	83.00±2.2
7	61.0±2.3	15.00±1.2	-	-	-	-	74.00±3
8	55.0±3.2	-	-	-	-	-	66.00±2.8

Annex 3. Mean percentage protection of the essential oils at 10% concentration of the test plants against *Ae. aegypti*.

Hours post application	% protection (Mean \pm S.E)						
	<i>O. suave</i>	<i>L. adoensis</i>	<i>S. molle</i>	<i>E. citriodora</i>	<i>E. globulus</i>	<i>O. lamiifolium</i>	DEET
1	100.00	83.6 \pm 1.6	55.3 \pm 2.9	29.33 \pm 3.6	30.00 \pm 9.2	46.00 \pm 6.6	100.0 \pm 0
2	97.66 \pm 2.3	63.0 \pm 1.1	29.66 \pm 3.3	9.00 \pm 4.5	11.33 \pm 6.1	19.66 \pm 3.5	100.0 \pm 0
3	88.66 \pm 1.8	49.33 \pm 2.9	14.00 \pm 4.1	0.00 \pm 0	0.00 \pm 0	9.66 \pm 2.6	100.0 \pm 0
4	75.00 \pm 2.0	35.00 \pm 1.5	6.00 \pm 3.0	0.00 \pm 0	0.00 \pm 0	0.00 \pm 0	98. \pm 1.3
5	61.66 \pm 2	24.00 \pm 0.5	0.00 \pm 0	0.00 \pm 0	0.00 \pm 0	0.00 \pm 0	93.00 \pm 2
6	50.00 \pm 2.2	8.00 \pm 1.1	-	-	-	-	84 \pm 0.2
7	40.00 \pm 1.3	-	-	-	-	-	75 \pm 2.3
8	30.00 \pm 2.7	-	-	-	-	-	67 \pm 3.1

Annex 4. Mean percentage protection of the essential oils at 20% concentration of the test plants against *Ae. aegypti*.

Hours post application	% protection (Mean \pm S.E)						
	<i>O. suave</i>	<i>L. adoensis</i>	<i>S. molle</i>	<i>E. citriodora</i>	<i>E. globulus</i>	<i>O. lamiifolium</i>	DEET
1	100.00 \pm 0	93.66 \pm 3.2	62.0 \pm 2.6	61.33 \pm 5.9	40.00 \pm 2	59.33 \pm 3.8	100.0 \pm 0
2	100.00 \pm 0	77.66 \pm 4.4	36.66 \pm 5.5	40.00 \pm 2.0	22.66 \pm 2.9	34.33 \pm 4.3	100.0 \pm 0
3	100.00 \pm 0	64.00 \pm 2.3	22.0 \pm 4.5	22.33 \pm 2.4	8.00 \pm 0	19.33 \pm 3.5	100.0 \pm 0
4	95.33 \pm 0.3	51.33 \pm 1.3	11.33 \pm 0.6	7.66 \pm 1.4	0.00 \pm 0	4.66 \pm 3.7	98. \pm 1.3
5	85.66 \pm 0.6	36.00 \pm 2.5	1.00 \pm 0.5	0.00 \pm 0	0.00 \pm 0	0.00 \pm 0	93.0 \pm 2
6	75.00 \pm 0.9	10.00 \pm 1.2	-	-		-	84 \pm 0.2
7	65.00 \pm 1.2	-	-	-		-	75 \pm 2.3
8	56.00 \pm 3.2	-	-	-		-	67 \pm 3.1



Annex 5. Mean percentage protection versus time (in hour) provided by combination of essential oils of the test plants and the standard DEET against *Anopheles arabiensis* at 10% concentration.

Key: 1. *O. s* = *O. suave*

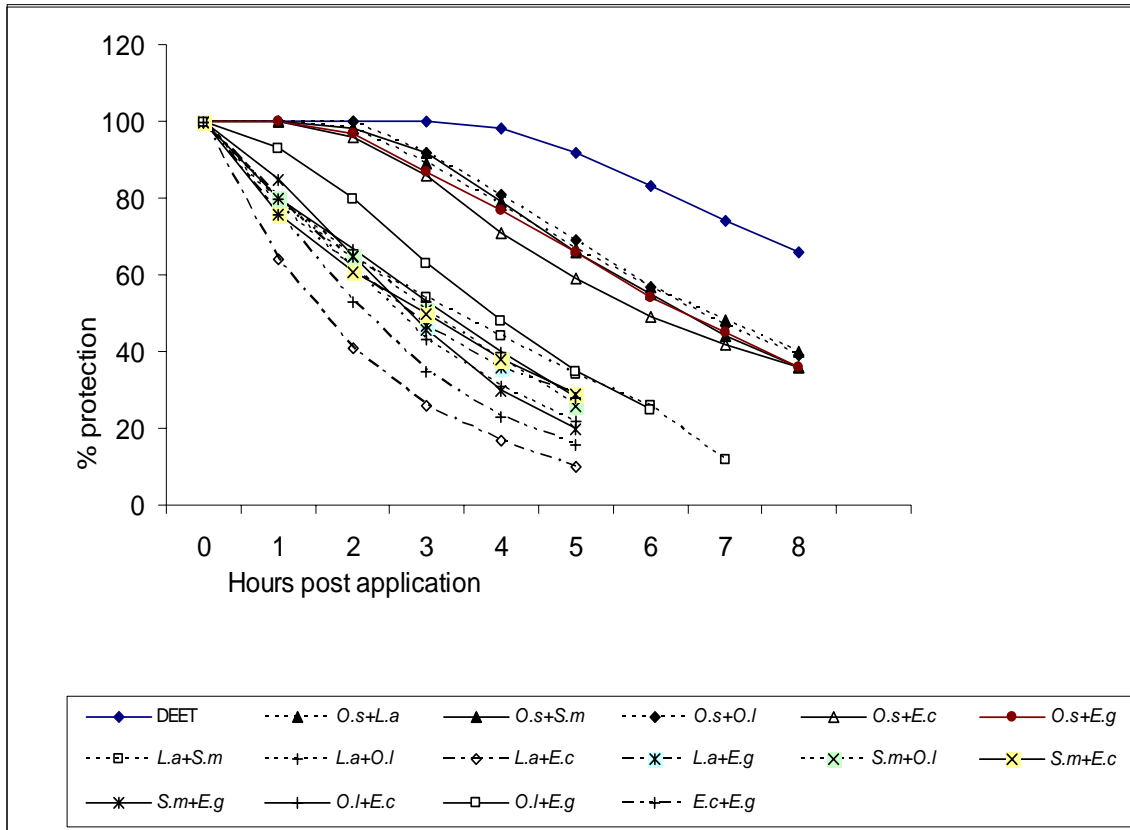
2. *L. a* = *L. adoensis*

3. *E. c* = *E. citriodora*

4. *S. m* = *S. molle*

5. *E. g* = *E. globulus*

6. *O. l* = *O. lamiifolium*



Annex 6. Mean percentage protection versus time (in hour) provided by combination of essential oils of the test plants and the standard DEET against *Anopheles arabiensis* at 20% concentration.

Key: 1. *O. s* = *O. suave*

2. *L. a* = *L. adoensis*

3. *E. c* = *E. citriodora*

4. *S. m* = *S. molle*

5. *E. g* = *E. globulus*

6. *O. l* = *O. lamiifolium*

Annex 7. Tukey HSD homogenous test at different hours of the test oils at 10% concentration against *An. arabiensis*.

10% concentration of test oils	1 hour	2 hour	3 hour	4 hour
	Alpha=0.05	Apha=0.05	Alpha=0.05	Alpha=0.05
<i>S. molle</i>	49.6a	29.3a	13.0a	9.6a
<i>E. globulus</i>	50.0a	28.6a	18.0a	11.3a
<i>L. adoensis</i>	56.3a	38.6a	29.0a	19.6a
<i>O.lamiifolium</i>	61.0a	40.0a	23.0a	10.3a
<i>E.citriodoa</i>	69.0a	48.3a	28.0a	18.0a
<i>O. suave</i>	83.3b	69.0b	56.0b	44b
DEET	100b	100c	100c	97.6c
P-value	0.055	0.56	0.089	0.091

Mean percentages of the essential oils followed by the same letters at each hour were not significantly different ($p>0.05$).

Annex 8. Tukey HSD homogenous test at different hours of the test oils at 20% concentration against *An. arabiensis*.

20% conc.	1 hour	2 hour	3 hour	4 hour	5 hour
	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05
<i>S. molle</i>	59.3a	40.0a	27.6a	16.0a	8.3a
<i>E. globulus</i>	60.3a	42.6a	25.0a	15.6a	9.0a
<i>E. citriodora</i>	65.6a	47.6a	36.6a	26.3a	11.6a
<i>O. lamiifolium</i>	77.3a	57.0a	44b	27.3a	13.6a
<i>L. adoensis</i>	100b	89.6b	77.3c	66b	50b
<i>O. suave</i>	100b	95.0b	89.3c	83c	77c
DEET	100b	100b	100c	97.6c	92c
P-value	0.055	0.56	0.08	0.09	0.82

Mean percentage protection of the essential oils followed by the same letters at each hour were not significantly different ($p>0.05$).

Key: HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 9. Tukey HSD multiple homogenous test at different hours of the test oils at 10% and 20% concentration against *An. arabiensis*.

Test oils	1 hour	2 hour	3 hour	4 hour	5 hour
	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05
10%Sm	49.6a	29.3a	13.6a	9.6a	6a
10%Eg	50.0a	28.6a	18.6a	11.3a	6a
10%La	56.3a	38.6a	29.6a	19.6a	10a
20%Sm	59.0a	40.0a	27.6a	16.0a	8a
20%Eg	60.3a	42.6a	25.0a	15.6a	9a
10%Ol	61.0a	40.0a	23.0a	10.3a	4a
10%Ec	65.6a	47.6a	28.6a	18.0a	8a
20%Ec	69.3a	48.3a	36.6a	26.3a	11a
20%Ol	77.3b	57.0a	44b	27.3a	13a
10%Os	83.3b	69.0b	56.3b	44b	31.3b
20%La	100b	89.6c	77.3c	66b	50.3b
20%Os	100b	95.0c	89.3c	83c	77c
DEET	100b	100c	100c	97c	92c
p-value	0.055	0.09	0.09	0.09	0.82

Mean percentage protection of the essential oils followed by the same letters at each hour were not significantly different ($p>0.05$).

- Key:
- | | |
|-----------------------------------|--------------------------------------|
| 1. <i>Sm</i> = <i>S. molle</i> | 4. <i>Ol</i> = <i>O. lamiifolium</i> |
| 2. <i>Eg</i> = <i>E. globules</i> | 5. <i>Ec</i> = <i>E. citriodora</i> |
| 3. <i>La</i> = <i>L. adoensis</i> | 6. <i>Os</i> = <i>O. suave</i> |
7. HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 10. Tukey HSD homogenous test at different hours of the test oils at 10% concentration against *Ae. aegypti*.

Test oils	1 hour	2 hour	3 hour	4 hour
	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05
<i>E. citriodora</i>	29a	9a	0a	0a
<i>E. globulus</i>	30a	11a	0a	0a
<i>O. lamiifolium</i>	46b	19a	9a	0a
<i>S. molle</i>	55b	29.66b	14b	6a
<i>L. adoensis</i>	83c	63b	49.3c	35b
<i>O. suave</i>	100c	97c	88d	75c
DEET	100c	100c	100d	98d
P-value	0.2	0.3	0.3	0.1

Mean percentage protection followed by the same letters at each hour were not significantly different ($p>0.05$)

Annex 11. Tukey HSD homogenous test at different hours of the test oils at 20% concentration against *Ae. aegypti*.

<i>Test oils</i>	1 hour	2 hour	3 hour	4 hour	5 hour
	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05
<i>E. globulus</i>	40a			0a	0a
<i>O. lamiifolium</i>	59b	34a	19a	4a	0a
<i>E. citriodora</i>	61b		22a	7a	0a
<i>S. molle</i>	62b	36a	22a	6a	1a
<i>L. adoensis</i>	93c	77.6b	64b	51.3b	36b
<i>O. suave</i>	100c	100c	100c	95c	85c
DEET	100c	100c	100c	98c	93c
P-value	0.2	0.3	0.3	0.1	1

Mean percentage protection followed by the same letters at each hour were not significantly different ($p>0.05$)

Key: HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 13. Tukey HSD homogenous test for 1:1 combinations of the test oils at 10% concentration against *An. arabiensis*

Test oils	1 hour	2 hour	3 hour	4 hour	5 hour
	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05
<i>La+Ec</i>	63a	38a	25a	16a	3a
<i>La+Sm</i>	64a	45a	30a	19a	10a
<i>La+Ol</i>	64a	45a	31a	18a	8a
<i>Eg+Ol</i>	64a	46a	32a	20a	12a
<i>Ec+Ol</i>	65a	51a	37a	25a	15a
<i>Ec+Eg</i>	66a	41a	27a	15a	5a
<i>Ec+Sm</i>	68a	45a	29a	21a	10a
<i>La+Eg</i>	72a	50a	32a	21a	7a
<i>Eg+Sm</i>	76a	59b	43b	30a	15a
<i>Sm+Ol</i>	79b	60b	41b	29a	12a
<i>Os+Ec</i>	90c	73b	55b	42b	32b
<i>Os+Eg</i>	92c	78c	65b	53b	42b
<i>Os+La</i>	94c	92c	75c	59c	49b
<i>Os+Ol</i>	95c	88c	80d	70c	59bc
<i>Os+Sm</i>	100c	83c	74c	61c	52bc
DEET	100c	100d	100d	97d	92d
p-value	.13	.2	.22	.053	.3

Mean percentage protection followed by the same letters at each hour were not significantly different ($p>0.05$)

- Key:
1. *La* = *L. adoensis*
 2. *Sm* = *S. molle*
 3. *Ec* = *E. citriodora*
 4. *Eg* = *E. globules*
 5. *Ol* = *O. lamiifolium*
 6. *Os* = *O. suave*
 7. HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 14. Tukey HSD homogenous test for 1:1 combinations of the test oils at 20% concentration against *An. arabiensis*

Test oils	1 hour	2 hour	3 hour	4 hour	5 hour
	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05	alpha=0.05
<i>La+Ec</i>	64a	40.6a	26.6a	16.6a	10a
<i>La+Sm</i>	76a	61ab	54.3ab	44ab	35.6ab
<i>Ec+Eg</i>	77a	51ab	32a	23a	15a
<i>La+Ol</i>	78a	61ab	42ab	30.3a	12a
<i>La+Sm</i>	79a	64ab	54ab	44ab	35ab
<i>Ec+Ol</i>	80a	67ab	54ab	40ab	28ab
<i>Sm+Ol</i>	80a	64ab	51ab	38ab	28ab
<i>La+Eg</i>	80a	62ab	47ab	36ab	30ab
<i>Eg+Sm</i>	85.3ab	65ab	46ab	29.6a	21a
<i>Eg+Ol</i>	94b	80b	64ab	47ab	35ab
<i>Os+La</i>	100b	97c	88b	77b	66c
<i>Os+Ec</i>	100b	96c	85b	72b	59c
<i>Os+Eg</i>	100b	97c	87b	76b	67c
<i>Os+Sm</i>	100b	97c	91b	79b	66c
<i>Os+Ol</i>	100b	100c	91b	81b	69c
DEET	100b	100c	100b	97c	92d
p-value	.13	.052	.09	.55	.3

Mean percentage protection followed by the same letters at each hour were not significantly different ($p > 0.05$)

- Key:
- | | |
|-------------------------------------|------------------------------------|
| 1. <i>La</i> = <i>L. adoensis</i> | 2. <i>Eg</i> = <i>E. globules</i> |
| 3. <i>Sm</i> = <i>S. molle</i> | 4. <i>Ol</i> = <i>O. lamiifoli</i> |
| 5. <i>Ec</i> = <i>E. citriodora</i> | 6. <i>Os</i> = <i>O. suave</i> |

7. HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 15. Tukey HSD multiple homogenous tests for 10% and 20% concentration of the test oils against *An. arabiensis*.

Test oils	1 Hour	2 Hour	3 Hour	4 Hour
	Alpha=0.05	Alpha=0.05	Alpha=0.05	Alpha=0.05
10% <i>La</i> + <i>Ec</i>	63a	38a	25a	16a
10% <i>La</i> + <i>Sm</i>	64a	45a	30a	19a
10% <i>La</i> + <i>Ol</i>	64a	45a	31a	18a
10% <i>Eg</i> + <i>Ol</i>	64a	46a	32a	20a
20% <i>La</i> + <i>Ec</i>	64a	40a	26a	16a
10% <i>Ec</i> + <i>Ol</i>	65a	51ab	37a	25a
10% <i>Ec</i> + <i>Eg</i>	66a	41a	27a	53b
10% <i>Ec</i> + <i>Sm</i>	68a	45a	29a	21a
10% <i>La</i> + <i>Eg</i>	72ab	50ab	32a	21a
10% <i>Eg</i> + <i>Sm</i>	76ab	59b	43b	30a
20% <i>Ec</i> + <i>Sm</i>	76ab	61b	49b	37b
20% <i>Eg</i> + <i>Ec</i>	77ab	51ab	32a	23a
20% <i>La</i> + <i>Ol</i>	78b	60b	42b	30a
20% <i>La</i> + <i>Sm</i>	79b	64bc	54b	44b
10% <i>Sm</i> + <i>Ol</i>	79b	59b	41a	29a
20% <i>Ec</i> + <i>Ol</i>	80b	67c	54b	40b
20% <i>Sm</i> + <i>Ol</i>	80b	64bc	51b	38b
20% <i>La</i> + <i>Eg</i>	80b	62b	47b	36b
20% <i>Eg</i> + <i>Sm</i>	85bc	65c	46b	29a
10% <i>Os</i> + <i>Ec</i>	90bc	73c	55b	42b
10% <i>Os</i> + <i>Eg</i>	92bc	78c	65c	42b
10% <i>Os</i> + <i>La</i>	94c	92cd	75c	59c
20% <i>Eg</i> + <i>Ol</i>	94c	80c	64c	47b
10% <i>Os</i> + <i>Ol</i>	95c	88d	80c	70c
20% <i>Os</i> + <i>La</i>	100c	97d	88d	77c
20% <i>Os</i> + <i>Ec</i>	100c	96d	85d	72c
20% <i>Os</i> + <i>Eg</i>	100c	97d	87d	72c
20% <i>Os</i> + <i>Sm</i>	100c	97d	91d	79c
20% <i>Os</i> + <i>Ol</i>	100c	100d	91d	81c
10% <i>Os</i> + <i>Sm</i>	100c	83d	74c	61c
DEET	100c	100d	100d	97d
P-value	.09	.29	.07	.053

Mean percentage protection followed by the same letters at each hour were not significantly different ($p>0.05$)

- Key:
- | | |
|-------------------------------------|--------------------------------------|
| 1. <i>La</i> = <i>L. adoensis</i> | 4. <i>Eg</i> = <i>E. globulus</i> |
| 2. <i>Sm</i> = <i>S. molle</i> | 5. <i>Ol</i> = <i>O. lamiifolium</i> |
| 3. <i>Ec</i> = <i>E. citriodora</i> | 6. <i>Os</i> = <i>O. suave</i> |
7. HSD = Honestly Significantly Different (Tukey's Studentized test)

Annex 16 Mean percentage repellency \pm SE of essential oils in 30 seconds exposure against *An. arabiensis* at different doses.

Essential oils	% Repellency of essential oils at different doses			
	1%	2%	5%	7%
<i>O. suave</i>	40 \pm 2.2	59 \pm 6.5	77.2 \pm 4.4	100 \pm 0
<i>L. adoensis</i>	1%	2%	4%	5%
	37.5 \pm 11	66.66 \pm 8.8	85.71 \pm 1.7	100 \pm 0
<i>E. citriodora</i>	2%	5%	7%	10%
	40.74 \pm 12.2	57.69 \pm 2.9	89.28 \pm 7.5	100 \pm 0
<i>E. globulus</i>	36.66 \pm 9.9	55.55 \pm 2.8	73.33 \pm 6.6	100 \pm 0
<i>S. molle</i>	23.52 \pm 2.3	62.85 \pm 7.3	80 \pm 4.9	100 \pm 0
<i>O. lamiifolium</i>	18.75 \pm 3.5	44.44 \pm 5	78.94 \pm 9.1	100 \pm 0

Annex 17 Mean percentage repellency \pm SE of essential oils in 30 seconds exposure against *Ae. aegypti* at different doses

Essential oils	% Repellency of essential oils at different doses			
	1%	2%	5%	8%
<i>O. suave</i>	40 \pm 5	62.63 \pm 8.9	81.81 \pm 3.4	100 \pm 0
<i>L. adoensis</i>	1%	2%	5%	7%
	17.64 \pm 10	47.61 \pm 4.3	77.77 \pm 6.2	100 \pm 0
<i>E. citriodora</i>	2%	5%	7%	10%
	21.42 \pm 5.1	42.1 \pm 7.3	78.26 \pm 9.8	100 \pm 0
<i>E. globulus</i>	26.31 \pm 3.3	55 \pm 10.4	86.95 \pm 6.6	100 \pm 0
<i>S. molle</i>	47.05 \pm 4.5	72 \pm 5.9	90.47 \pm 10	100 \pm 0
<i>O. lamiifolium</i>	21.48 \pm 14	57.14 \pm 7.7	87.5 \pm 1.2	100 \pm 0

Annex 18 ANOVA table for 10% concentration of essential oils at different times against *An. arabiensis*

		Sum of squares	df	Mean squares	F	Sig.
1 hour	Between groups	6031.143	6	1050.190	7.801	0.001
	Within groups	1884.667	14	134.619		
	Total	8185.810	20			
2 hour	Between groups	1916.476	6	1986.079	15.992	0.0
	Within groups	1738.667	14	124.190		
	Total	13655.143	20			
3 hour	Between groups	16575.810	6	2762.635	43.984	0.0
	Within groups	879.333	14	62.810		
	Total	17455.143	20			
4 hour	Between groups	18522.476	6	3087.079	63.620	0.0
	Within groups	679.333	14	48.524		
	Total	19201.810	20			
5 hour	Between groups	18305.143	6	3050.857	94.355	0.0
	Within groups	452.667	14	32.333		
	Total	18757.810	20			

Annex 19 ANOVA table for 20% concentration of essential oils at different times against *An. arabiensis*

		Sum of squares	df	Mean squares	F	Sig.
1 hour	Between groups	6718.667	6	1119.778	36.800	0.0
	Within groups	426.000	14	30.429		
	Total	7144.667	20			
2 hour	Between groups	12541.143	6	2090.190	41.924	0.0
	Within groups	698.000	14	49.857		
	Total	13239.143	20			
3 hour	Between groups	17323.905	6	2887.317	42.972	0.0
	Within groups	940.667	14	67.190		
	Total	18264.571	20			
4 hour	Between groups	20938.476	6	3489.746	118.969	0.0
	Within groups	410.667	14	29.333		
	Total	21349.143	20			
5 hour	Between groups	22828.286	6	3804.714	111.747	0.0
	Within groups	476.667	14	34.048		
	Total	23304.952	20			

Annex 20 ANOVA table for 10% concentration of essential oils at different times against *Ae. aegypti*

		Sum of squares	df	Mean squares	F	Sig.
1 hour	Between groups	17201.238	6	2866.873	43.344	0.0
	Within groups	926.000	14	66.143		
	Total	18127.238	20			
2 hour	Between groups	18186.571	6	4697.762	124.405	0.0
	Within groups	528.667	14	37.762		
	Total	28715.238	20			
3 hour	Between groups	32410.952	6	5401.825	346.906	0.0
	Within groups	218.000	14	15.571		
	Total	32628.952	20			
4 hour	Between groups	30114.000	6	5019.000	658.744	0.0
	Within groups	106.667	14	7.619		
	Total	30220.667	20			
5 hour	Between groups	25537.810	6	4256.302	1160.810	0.0
	Within groups	51.333	14	3.667		
	Total	25589.143	20			

Annex 21 ANOVA table for 20% concentration of essential oils at different times against *Ae. aegypti*

		Sum of squares	df	Mean squares	F	Sig.
1 hour	Between groups	10218.571	6	1703	55.622	0.0
	Within groups	428.667	14	30.619		
	Total	10647.238	20			
2 hour	Between groups	19495.143	6	3249.190	92.332	0.0
	Within groups	492.667	14	35.190		
	Total	19987.810	20			
3 hour	Between groups	28261.619	6	4710.270	243.397	0.0
	Within groups	281.333	14	20.095		
	Total	28542.952	20			
4 hour	Between groups	33991.143	6	5665.190	660.939	0.0
	Within groups	120.000	14	8.571		
	Total	34111.143	20			
5 hour	Between groups	32045.238	6	5340.873	1110.479	0.0
	Within groups	67.333	14	4.810		
	Total	32112.571	20			

Annex 22 ANOVA table for 10% concentration of blends of the essential oils at different times against *An. arabiensis*

		Sum of Squares	df	Mean Square	F	Sig.
1 hour	Between groups	9357.000	15	623.800	48.687	.000
	Within groups	410.000	32	12.813		
	Total	9767.000	47			
2 hour	Between groups	18767.250	15	1251.150	82.721	.000
	Within groups	484.000	32	15.125		
	Total	19251.250	47			
3 hour	Between groups	24354.000	15	1623.600	173.184	.000
	Within groups	300.000	32	9.375		
	Total	24654.000	47			
4 hour	Between groups	26331.250	15	1755.417	163.930	.000
	Within groups	342.667	32	10.708		
	Total	26673.917	47			
5 hour	Between groups	29173.250	15	1944.883	215.599	.000
	Within groups	288.667	32	9.021		
	Total	29461.917	47			

Annex 23 ANOVA table for 20% concentration of blends of the essential oils at different times against *An. arabiensis*

		Sum of squares	df	Mean Square	F	Sig.
1 hour	Between Groups	6132.583	15	408.839	12.911	.000
	Within Groups	1013.333	32	31.667		
	Total	7145.917	47			
2 hour	Between Groups	17692.646	15	1179.510	33.660	.000
	Within Groups	1121.333	32	35.042		
	Total	18813.979	47			
3 hour	Between Groups	25581.250	15	1705.417	40.706	.000
	Within Groups	1340.667	32	41.896		
	Total	26921.917	47			
4 hour	Between Groups	27615.250	15	1841.017	54.414	.000
	Within Groups	1082.667	32	33.833		
	Total	28697.917	47			
5 hour	Between Groups	27496.812	15	1833.121	50.627	.000
	Within Groups	1158.667	32	36.208		
	Total	28655.479	47			



E. citriodora



E. globulus



L. adoensis



O. lamiifolium



O. suave



S. molle

Annex 24. The test plants (Source: Fichtl and Adi (1994)).



Annex 25. Essential oil extraction apparatus



Annex 26. Human volunteers exposing arms in the cages

DECLARATION

This thesis is my original work and has not been presented for a degree in any university and that all sources of materials used for the thesis have been duly acknowledged.

Name Mulugeta Wano

Signature _____

Date July, 2006