



ADDIS ABABA UNIVERSITY
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Evaluation of the repellent activity of essential oils extracted from some selected medicinal plants in Ethiopia against Adult *Anopheles arabiensis* Patton (Diptera: culicidae) under laboratory conditions

BY

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Postgraduate Program
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Evaluation of the repellent activity of essential oils extracted from some selected medicinal plants in Ethiopia against Adult *Anopheles arabiensis* Patton (Diptera: culicidae) under laboratory conditions

By

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A thesis Submitted to the Graduate Programme of the Addis Ababa University for the partial fulfillment of the requirements for the Degree of Master of Science in Zoological Sciences (Insect Sciences)

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APPROVAL SHEET

As thesis advisors, we, hereby, certify that we have read and evaluated the thesis entitled “Evaluation of the repellent activity of selected medicinal plants in Ethiopia against Adult *Anopheles arabiensis* Patton (Diptera: culicidae)” prepared under our supervision by Tilanew Getie. We recommend the thesis to be submitted for defense as it fulfills the required standard of the level.

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LIST OF ACRONYMS

AIDS– Acquired immunodeficiency syndrome

DDT - Dichloro-diphenyl-trichloroethane

DEET–N, N-diethyl-M- methyl-benzaamide

ITNs – Insecticides treated mosquito nets

MoH – Minister of Health

WAFC - World Agroforestry Centre

WHO – World Health Organization

ED 50 – Effective Dose for 50% of the population

ED 95 – 95% effective dose for 95% of the population

EOS – Essential oils

ABSTRACT

Mosquitoes are vectors of several important pathogens and parasites, including malaria, which is transmitted by a bite of an infected female mosquito of the genus Anopheles. Malaria remains the leading cause of morbidity and mortality around the world mainly in sub-Saharan African countries including Ethiopia. Even though different controlling methods of malaria vectors have been used, it becomes more difficult to malaria expansion due to the rapid resistance development of mosquitoes to insecticides and synthetic repellents. Toxic reactions of the chemicals have also been reported in some circumstances. Therefore, this study was designed to evaluate the repellent efficacy of six selected traditionally used medicinal plants including Citrus limon, Kleinia odora, Securidaca longepedunculata, Warburgia ugandensis, Rosmarinus officinalis, and Lavandula angustifolia as repellents against Anopheles arabiensis in Ethiopia. Also, to evaluate dermal and oral toxicity on animals. Plant parts were collected from different parts of the country. Essential oils (EOs) were extracted using Clevenger hydro distillation by heating at 120 °C. Serial dilutions of each plant repellent were made with ethanol and tested to identify an effective dose range and tested on volunteers using laboratory-reared An. arabiensis. 5% and 10% (W/V) ointment were formulated by combining 0.25 and 0.5 mls of EOs in 5 g of petroleum jelly respectively for skin irritation assessment. Skin irritation was examined on adult guinea pigs using half a gram of 5% and 10% EO ointment of W.ugandensis since it was found to have the best repellent effect. Acute oral toxicity was also tested on albino mice at different concentrations of W. ugandensis essential oils. At 5% (W/V) concentration, W. ugandensis showed lowest ED₅₀ (0.019%) and ED₉₅ (0.410%) values against An. arabiensis showing the highest repellent activity among the candidate plants. While, S. longepedunculata scored the least repellent power. However, no significant difference was observed in all plants for both doses. Essential oils of all plants had >89% mosquito repellent ability for 2.5 hours and W. ugandensis lasted its complete repellent power for up 4 hours. Skin irritation assessment of W. ugandensis essential oils showed the absence of any dermal effect. However, an acute oral toxicity test showed the presence of some symptoms like diarrhea, drowsiness, and erection of fur starting from the doses of 2400 mg/Kg body weight of mice, and death was observed at 4800 mg/Kg seven days after treatment. Significant body weight loss was also observed at the dose of 4800 mg/Kg. This showed a slight toxicity effect of the oils extracted from the leaves of the plant. In general, essential oils from all the plants under study showed to have remarkable mosquito repellent power although variation was observed in between. Possibilities to stabilize the essential oils for their prolonged repellent effect should be developed by changing the type of solvents used. Further studies on the optimization of doses for repellency tests and does for skin irritation should be conducted.

Key Words: *An. arabiensis*, Essential oils, Malaria, Mosquito repellents

1. INTRODUCTION

1.1. Background of the study

Mosquitoes are vectors of several important pathogens, including arboviruses, protozoans, and filariae that cause serious diseases of major public health concern (Tandina *et al.*, 2018). Among them, malaria remains one of the most devastating diseases occurring in the world today.

In sub-Saharan Africa, children under the age of five years and pregnant women are highly affected by malaria (Morlais *et al.*, 2005). Currently, malaria threatens almost one-third of the world's population in 84 tropical countries and territories where it is considered an endemic disease (WHO, 2022). The World Health Organization (WHO) estimates that 247 million cases of malaria occurred globally in 2021 and led to 625,000 deaths (WHO, 2022).

In many regions of the world, mosquito-borne diseases are flourishing particularly in this era of climate change, and are responsible for significant global morbidity and mortality. Africa, South-East Asia, and the Eastern Mediterranean were the regions with the highest numbers of reported cases and deaths, mainly in children less than five years of age (WHO, 2022).

Nearly 60 percent of Ethiopia's population lives in malarious areas, and 75 percent of the country's land mass is favorable for malaria transmission (MoH, 2017). The disease has been regularly reported as one of the top three leading causes of outpatient visits, admissions, and deaths among all age groups in Ethiopia (Berhe *et al.*, 2019).

In different parts of the world including Africa, communities are using different personal protection measures to prevent mosquito bites (Anand *et al.*, 2014). However, the method is not environmentally safe, biodegradable, and cost-effective against malaria vector. Mosquito repellents are currently used complementarily with other integrated vector control options (Katz *et al.*, 2008; Tyagi, 2016; Maia *et al.*, 2018). However, repellents are not safe for humans, especially children because they may cause skin irritation, hot sensation rashes, or allergy (Das *et al.*, 2003), such as DEET (N, N-diethyl-M-methyl benzamide) and IR3535 (ethyl butylacetylaminopropionate) may be unsafe for children possibly causing encephalopathy (Abdle-Rahman *et al.*, 2001; Menon and Brown, 2004). Consequently, here is an urgent need to develop new repellents for controlling mosquito vectors that are more environmentally friendly, biodegradable, non-toxic effects on humans and domestic animals (Kumar *et al.*, 2011; Rabha *et*

al., 2012; Gnankiné and Bassoe, 2017). The traditional use of plant products as natural repellents and insecticides is of great interest in the search for new active substances for vector control.

In connection to this, this study is designed to investigate the repellency of essential oils derived from some selected traditional medicinal plants found in Ethiopia against *An. arabiensis* under laboratory conditions.

1.2. Statement of the problem

The control of mosquito vectors has become more difficult because some mosquitoes have rapidly become resistant to insecticides and synthetic repellents (Wagman *et al.*, 2015; The behavior of the vector mosquitoes and the human host has made changes for mosquitoes increasingly biting outside (Degefa *et al.*, 2021) and human activities extend towards late night where mosquitoes are active., Cuervo-Parra *et al.*, 2016). N, N-Diethyl-m-methyl benzamide (DEET) has been considered as one of the most effective synthetic repellents against mosquitoes. However, its toxic reactions have been reported in some circumstances, especially among children and the elderly people (Wanzala *et al.*, 2014). In addition, significant amounts of this chemical can be absorbed through the skin. DEET also acts as a plasticizer and has an objectionable odor to some individuals. In general, concerns have been raised over the risk of adverse toxic effects, especially in young children and pregnant and lactating women (Mc Gready *et al.*, 2001; Osimitz *et al.*, 2010). In compensation to this, essential oil-derived compounds from the plant, with a few exceptions, can be applied to humans in a similar way to other conventional insecticides and they tend to be selective and have little or no harmful effects (Sharifi-Rad *et al.*, 2017). But not all plant species are tested for their insect repellency effect. So, conducting a research and development of alternative repellent against mosquitoes with lower or no toxicity to humans are highly desired. In this study, some plant species that have insecticide potential and have not been tested for their repellency activity against malaria vectors in Ethiopia were selected.

1.3. Objective

1.3.1. General objective

- Assessing the repellent efficacy of some selected traditionally used medicinal plants (*Citrus limon*, *Kleinia odora*, *Securidaca longepedunculata*, *Warburgia ugandensis*,

Rosmarinus officinalis, and *Lavandula angustifolia*) as repellents against *Anopheles arabiensis* and conduct toxicity study in Ethiopia.

1.3.2. Specific objectives

- To evaluate the relative effectiveness of repellency of the essential oils of different plant species.
- To estimate dose-response lines and effective doses (EDs) of a repellent corresponding to 50% (ED50) and 95% (ED95) protection from mosquito landing and/or probing.
- To estimate the complete protection time (CPT) of a repellent
- To conduct acute toxicity study and skin irritation tests of the repellent on experimental animal

1.4. Significant of the study

The findings of this study could help in malaria control programs, particularly by reducing human mosquito contact; thereby reducing the prevalence, intensity, and transmission of malaria. In addition, the findings could providevaluable data for the production of environmentally safe potential repellents against malaria vectors from traditional medicinal plants in Ethiopia.

2. LITERATURE REVIEW

2.1. Global malaria burden

Mosquito-borne diseases contribute significantly in tropical countries to the burden of illness, death, hunger, and social vulnerability. Among these diseases, malaria has a major impact on global public health and the economy, with an estimated 3.4 billion people at risk (Cibulskis *et al.*, 2016). Currently, malaria threatens almost one-third of the world's population in 84 tropical countries and territories where it is considered an endemic disease (WHO, 2022). The World Health Organization estimates that 234 million cases of malaria occurred globally in 2021 and led to 625,000 deaths. Africa, South-East Asia, and the Eastern Mediterranean were the regions with the highest numbers of reported cases and deaths reported, mainly in children less than five years of age (WHO, 2022).

Malaria is caused by a parasitic protozoan infection of the genus *Plasmodium*. Several *Plasmodium* species infect humans and other animals, including birds, reptiles and rodents (Alexandre *et al.*, 2010; Costa *et al.*, 2012). *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* are the major species of *Plasmodium* parasite in humans (Nicoletti, 2020). *P. falciparum* is the most common prevalent species in sub-Saharan African countries including Ethiopia.

According to WHO (2018) reports, *P. falciparum* is the most prevalent malaria parasite in the African Region, accounting for 99.7% of estimated malaria cases in 2018, as well as in the South-East Asia Region (50%), the Eastern Mediterranean Region (71%) and the Western Pacific Region (65%). Globally, 53% of the *P. vivax* burden is in the WHO South-East Asia Region, with the majority (47%) being in India (WHO, 2019). *P. vivax* is the predominant parasite in the Region of the Americas, representing 75% of malaria cases (WHO, 2019).

2.1.1. Malaria burden in Africa

The burden of malaria was higher in the African region, where around 93% or 213 million cases of malaria were observed in 2018 alone (WHO, 2018). Malaria deaths are reduced by 44%, from 680 000 in 2000 to 384 000 in 2019, and the malaria mortality rate reduced by 67% over the same period, from 121 to 40 deaths per 100 000 population at risk (WHO, 2020). Central Africa had the highest prevalence of exposure to malaria during pregnancy (40%), closely followed by

West Africa (39%), while prevalence was 24% in East and Southern Africa. In 2019, 6 countries accounted for approximately half of all malaria deaths worldwide: Nigeria (23%), the Democratic Republic of the Congo (11%), United Republic of Tanzania (5%), Burkina Faso (4%), Mozambique (4%) and Niger (4%) from Africa country (WHO, 2019).

The economic burden of malaria is immense for the nation, the family, and the individual. It has been estimated that the annual per capita economic growth rate in malaria-endemic countries has decreased by 1% (Sicuri *et al.*, 2013).

In particular, the economic impacts of malaria are visible in rural areas where malaria strikes at the time of the year when agricultural work is most needed (Killeen, 2014). The situation is worsening with the evolution of resistance to cheap and easily available drugs and insecticides, changes in environmental conditions leading to increasing epidemics, civil unrest coupled with population movements, and economic development programs in risk areas such as wetlands, and desert fringes, and highlands. Indeed malaria has spread into areas, which previously had low or no transmission (Killeen, 2014).

2.1.2. Malaria burden in Ethiopia

Malaria transmission is highly affected by environmental variables such as topography, rainfall, climate, and socio-economic conditions of the population (Tesfaye *et al.*, 2011; MoH, 2012; Ayele *et al.*, 2013). For this reason, tropical regions including Ethiopia with warm temperatures, heavy rainfall, and high humidity are conducive to mosquito breeding, longevity, and parasite sporogony (Messina *et al.*, 2011). Ethiopia share great burden of malaria and is placed 15th from the highly malaria-affected countries in Africa (WHO, 2021). Thus, malaria continues to strike hardest against the health of the population in the country. In most parts of the country, the peak periods of malaria incidence occur from September to December, following the main rainy seasons (June-September), and from March to May, during and after the small rainy seasons from February-March (MoH, 2012; Andargie *et al.*, 2022). Three-fourths of the country is at risk of malaria, most of the lowland mass where more than 65% of the population lives (MoH, 2015). Approximately, 4–5 million cases of malaria and 70,000 related deaths have been reported annually in the previous years (MoH, 2015).

Although Ethiopia has targeted malaria elimination at this moment, several factors such as emergence of resistance of the parasite to antimalarials and vectors to insecticides, the

complexity of the control process in *P. vivax*, seasonal transmission with diverse micro-climates and low population immunity challenged malaria elimination in the country (USAID, 2020).

There was, in the country, a fluctuating trend of malaria prevalence within the last decade showing a significant reduction of malaria cases and deaths (USAID, 2017; Alkadir, 2020). In line to this, the report of Tadele *et al.* (2019) showed the decrease in the number of deaths due to malaria by 54% from the 2000's record of 10,412 deaths within 16 years and age-standardized death rate declining by 63% from the 2000 record. A significant decline in malaria has also been reported in Ethiopia (Solomon *et al.*, 2020) although unstable malaria transmission patterns make the country prone to epidemics (EPHI, 2016). This is because the decrease in malaria burden is not uniform in the country which is imperative to understand the contextual diversity of malaria prevalence within each site (Andargie *et al.*, 2022).

2.2 Malaria vectors

The malaria vectorial system is very complex. Mosquitoes of the *Anopheles* genus are the vectors of the *Plasmodium* species.. The species of *Anopheles* have a worldwide distribution, occurring in both tropical and temperate regions (Service, 2008). Recently, there are approximately 530 known species of *Anopheles*, but only 30-40 species are known to transmit malaria in nature (Nicoletti, 2020).

2.2.1. Malaria vectors in Africa

Members of the *An. gambiae* complex are the most important vectors of malaria in sub-Saharan Africa. The complex consists of about nine species: *An. gambiae s.s.*, *An. arabiensis*, *An. quadriannulatus* spp. A, *An. bwamambe*, *An. merus*, *An. melas* and *An. quadriannulatus* spp. B and also the two new species; *An. coluzzii* and *An. amharicus* (Coetzee *et al.*, 2020). Two species of the complex: *An. gambiae s.s.* and *An. arabiensis*, are both the most broadly distributed and the most efficient vectors of malaria in Africa (Wiebe *et al.*, 2017).

Both of them are strongly influenced by climatic factors, especially in total annual rainfalls. *An. arabiensis* tends to dominate in arid savannas, whereas *An. gambiae s.s.* is the dominant species in humid forest zones (Akpan *et al.*, 2019). However, in most of Africa, the two species occur in sympatry (North and Godfray, 2018). *An. funestus*, *An. bwamambe*, *An. merus*, and *An. melas* are also an important species in malaria transmission in localized areas (Sinka *et al.*, 2010).

2.2.2. Malaria vectors in Ethiopia

In Ethiopia, 44 *Anopheles* species have been known. But, *An. arabiensis* in the *An. gambiae* complex is the most common and primary vector (Abose *et al.*, 1998; WHO, 2016). *An. nili*, *An. pharoensis* and *An. funestus* are the second important vectors of malaria (MoH, 2007; WHO, 2016).

2.3. Biology and bionomics of *Anopheles arabiensis*

Anopheles arabiensis is a member of the *Anopheles gambiae* species complex and one of the most prominent malaria vectors in Sub-Saharan Africa (Coetzee *et al.*, 2013). According to the ecological niche modeling of Drake and Beier (2014), *An. arabiensis* is a climatic generalist, meaning it can survive in most of Africa's current environmental conditions. *An. arabiensis* species are reflected as r-strategist. They can settle on aquatic habitats within a few days of their creation because predators may be less prevalent in small temporary habitats, and larval food may be more plentiful than in long-term habitats, even though some research suggests that stable habitats are more productive than temporary unstable habitats (Munga *et al.*, 2013). *An. arabiensis* needs minuscule, temporary, sunny, clear, shallow fresh water pools and can use a varied range of habitats than *An. gambiae* s.s, involving slow-moving, partially shaded streams and a variety of small and big natural and man-made habitats (Sinka *et al.*, 2010).

It has also occurred in turbid waters, brackish habitats, and irrigated rice fields (Sinka *et al.*, 2010). Many aquatic biological activities, including the growth and development of mosquito immatures (*An. arabiensis* and *An. gambiae* s.l), are affected by water temperature (Paaijmans *et al.*, 2008).

The oviposition, survival, and spatial dispersion of mosquito vectors are affected by the physicochemical characteristics of mosquito breeding sites to some extent (Garba and Olayemi, 2015; Emidi *et al.*, 2017). Physicochemical parameters such as temperature, salinity, conductivity, total dissolved solids (TDS), and pH have major factor in the existence and larval copiousness among mosquito species (Oyewole *et al.*, 2009; Kwasi, 2012; Imam and Deeni, 2014).

The distribution and richness of malaria vectors are influenced by climatic factors such as moisture index and temperature (Fox and Westneat, 2010). The distribution of mosquito vectors

is determined to a significant extent by climatic conditions and species' habitats all over the world (Manguin, 2013).

2.4. Behavior of *Anopheles arabiensis*

Knowing the behavior of vector species, as well as their ecology and microclimate, is critical for determining the most effective management strategy (Ngowo *et al.*, 2017). Different mosquito species have different behavioral characteristics such as resting pattern, oviposition site selection, egg deposition, feeding habit, host preference, feeding time, specific part of the host on which they feed (Braack *et al.*, 2015; Lelisa *et al.*, 2017; Ngowo *et al.*, 2017).

2.4.1. Biting behavior

Understanding the biting activities of mosquitoes is very crucial for malaria control interventions (Sherrard-Smith *et al.*, 2019). Almost all anopheline species bite at night significantly while due to the application of IRS and LLINs, a shift in mosquito behaviors has been observed, where mosquitoes more often bite humans outdoors and earlier in the evening, thereby avoiding insecticide-treated surfaces and threatening the effectiveness of the interventions (Gatton *et al.*, 2013; Sokhna *et al.*, 2013). This behavioral change has been observed in Tanzania with *An. arabiensis* (Kitau *et al.*, 2012). High levels of outdoor biting by *An. gambiae* s.s were found on Bioko Island, Equatorial Guinea, throughout the night, including early evening and morning hours when human hosts are frequently outside (Overgaard *et al.*, 2012).

After the introduction of LLINs in Benin and Senegal, *An. funestus* changed its biting behavior, remaining anthropophilic and endophilic while adopting diurnal biting when indigenous people are not protected by IRS and LLINs (Moiroux *et al.*, 2012; Sougoufara *et al.*, 2014).

Due to the application of insecticides, the biting behavior of *An. arabiensis* has been changed in Africa (Fornadel *et al.*, 2010; Kitau *et al.*, 2012; Gatton *et al.*, 2013). In Zambia, for example, *An. arabiensis* bites throughout the night, with peak activity beginning at 10:00 p.m. In Senegal, Chad, and Kenya, peak biting has also been seen around midnight. Whereas, high biting activity was reported as early as 9:00 p.m. in Mozambique and Tanzania (Fornadel *et al.*, 2010). In Ethiopia, the biting activity of *An. arabiensis* occurred before 10 pm hours and thus before the time when people usually go to bed (Yohannes and Boelee, 2012). During the sleeping time

(4:00-11:00) indoor biting activity *An. arabiensis* and outdoor human-biting activities during the early night were observed in South-central Ethiopia (Kenea *et al.*, 2016).

2.4.2. Feeding and resting behavior

Female *Anopheles* mosquitoes bite humans to receive a blood meal, and a few prefer humans to animals. Whereas, some species of mosquitoes fed more or less extensively at any time of the day or night. Mosquitoes seek safe resting spots to hide for digesting their blood meal and their ovaries maturity. Some evidence shows that *An. arabiensis* and *An. funestus* were mainly endophagic and endophilic as compared to other *Anopheles* species such as *An. pharoensis*, *An. welcomi*, *An. zeimanni* and *An. nili*, which were mainly exophagic and exophilic (Taye *et al.*, 2006). Some mosquitoes exclusively feed on humans (anthropophagic) or animals (zoophagic), or some of them feed both on animals and humans depending on their availability (Randell *et al.*, 2010).

The mosquito can be classified as endophilic those which prefer to rest indoors and exophilic those which tend to rest outdoor (Gatton *et al.*, 2013), and thus, species vary in their preference of resting places. *An. arabiensis* which covers more than 70% of sub-Saharan Africa adapts to endophagic and endophilic patterns, where hosts are domestic and indoor, but adapts exophagic patterns where hosts are mainly outdoors.

Vectors are changing their feeding habits as a result of extensive vector control activities. The report of Sy *et al.* (2018) indicated that more aggressive outdoors biting of *An. arabiensis* in regions where IRS is applied in Senegal. Recently, *An. gambiae s.l.* also look for hosts in outdoor as much as indoors in the Punta Europa region of Bioko Island which compares with an earlier pre-intervention surveillance of exclusive endophagy of *An. gambiae* in this region (Reddy *et al.*, 2011).

It is believed to be due to the application of long-term indoor residual spraying (IRS) with insecticide (Reddy *et al.*, 2011). In Ethiopia, a reduced endophilic preference of *An. gambiaes.l.* were recorded after the application of IRS intervention (Lelisa *et al.*, 2017).

An. arabiensis and *An. funestus s.l.* were also reported in a different country with zoophilic and exophilic tendencies. In Kenya, for example, *An. gambiaes.s.* showed a dramatic change from endophilic to exophilic behavior after the application of LLIN use (Carnevale, and anguin,

2021). Similarly, outdoor behavior in *An. funestus* has also been observed in Benin, West Africa, where a great proportion of mosquito populations are active after dawn (Moiroux *et al.*, 2012).

Environmental factors can also influence mosquito resting behavior. For example, the resting behavior of *An. demeilloni* was shifted from exophilic in the lowland to endophilic in the highland. This indicated that temperature affects the biology of anophelines. Thus, they can escape the effect of low temperature and unstable humidity in high altitudes by resting indoors (Gone *et al.*, 2014).

2.4.3. Oviposition site selection behavior

Mosquitoes lay two kinds of eggs: Rapid-Hatch (RH) and Delayed-Hatch (DH). Rapid hatch eggs are laid directly into the water, on the water surface, or substrate close to the water and usually hatch within 48 hrs. The RH eggs are laid individually, in small groups, or rafts containing up to several hundred eggs.

Delayed Hatch eggs are usually laid singly or in small groups, are drought-resistant, survive for long periods out of the water, hatch soon after being refolded, and sometimes enter a photoperiod-induced diapause to survive temperate and arctic winters (Day, 2016).

Mosquitoes lay their eggs in a variety of watery niches, including natural ponds, puddles, stream fringes, marshes, treeholes, plant axils, man-made pits, drains, rice fields, and other containers. The selection of optimal oviposition locations is an important step in the mosquito's life cycle (Herrera-Varela *et al.*, 2014).

The interaction of a diverse array of chemical and physical variables leads to the selection of an oviposition site. Chemical cues can be detected before physical contact with the site, or they can be detected upon contact and may originate from a variety of sources, including microorganisms; mosquito eggs, larvae, or pupae; decomposing organic materials; microbes of larval breeding water, and predators or competitors, whether vertebrate or invertebrate (Himeidan *et al.*, 2013).

When a female mosquito has consumed a blood meal, physiological changes occur, causing it to change from host searching to oviposition site selection. Female mosquitos use visual, olfactory, gustatory and chemo-tactile signals to detect prospective oviposition sites. Among olfactory cues, female mosquitoes use pheromones and kairomomes to find an appropriate oviposition site (Wondwoson, 2016). *An. arabiensis* uses vegetation cues linked with larval habitats to select an

oviposition site. Identifying volatile cues from grasses that alter gravid malaria mosquito behavior gives a capacity for future monitoring and control strategies (Asmare *et al.*, 2017).

2.5. Malaria vector control

Vector control refers to measures of any kind against malaria-transmitting mosquitoes, intended to limit the ability or vectorial capacity to transmit the disease (WHO, 2016). Malaria eradication campaigns initiated in the 1950s and 1960s drastically reduced the proportion of the world population at risk of the disease from 68% in 1946 to 52% in 1975 (Snow *et al.*, 2012).

For a variety of vector-borne diseases, control of their vectors is the most efficient way to control their transmission. Vector control is an essential component of malaria prevention by aims at reducing the breeding and survival of mosquito vectors. The available vector control methods are chemical, biological, genetic, environmental management, personal protection, and integrated vector management. Although there are many vector control methods, most of them are too expensive, ecologically harmful, and environmentally unsafe or they are practically infeasible and inaccessible to be used in poor countries like Ethiopia. Moreover, insecticide resistance is now a major problem facing malaria vector control programs in most African countries, including Ethiopia, with the most important vector species, showing resistance to one or more of the insecticide classes used in vector control (Yewhalaw and Kweka, 2016).

2.5.1. Environmental management

Environmental management refers to the planning, organization, implementation, and monitoring of environmental factors modification and manipulation activities to prevent or minimize vector development and reducing contact between man-vector-pathogen (Randell *et al.*, 2010).

To minimize or eradicate mosquito-borne diseases, vector control experts have recognized the importance of modifying mosquito larval habitats. Efforts to remove or change habitat have included general initiatives to minimize the abundance of all mosquitoes. The environmental management for source reduction includes runoff, flushing, filling and making margins of rivers and lakes unfit for *Anopheles* breeding (WHO, 2013). Environmental managements are classical method of malaria vector control, which may be used for all mosquitoes breeding in general or targeted to the specific breeding places of malaria vectors of local importance (Yohannes *et al.*, 2005; Gari and Lindtjorn, 2018).

According to Yohannes *et al.* (2005) report, source reduction was carried out by the community resulting in a 49% relative reduction in *An. arabiensis* adults in the dam village in Tigray, Ethiopia compared with the pre-intervention period. Environmental managements are effective and have long-term effects. However, these methods have relatively high investment costs and may be cost-effective only in urban areas or some types of development projects.

2.5.2. Biological control

A biological technique of mosquito control fundamentally involves the use of living organism especially natural enemies (predators, parasites, or pathogens) of mosquitoes and biological toxoids to achieve effective control. Now a day, the principal biological control agents that have been successfully employed against mosquitoes are predators, particularly fish, and the bacterial pathogens *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* (Bs) that attack the larval stages of the mosquito (Benelli *et al.*, 2016).

Other promising species includes a variety of fungal pathogens, nematode (*Romanomermis culcivorax*), and Azolla aquatic plant (Walker and Lynch, 2007). Biological control methods have the advantage of target specificity with little effect on non-target organisms and are regarded as environmentally safe. However, the high cost and difficulties encountered in mass production preclude their wide use, especially in tropical countries.

2.5.3. Genetic control

The recent advances in insect genetic engineering have opened up new possibilities for genetically modifying insect vectors to reduce human diseases (Panjwani and Wilson, 2016). Several genetic methods of mosquito control are being studied under laboratory conditions, a few, containing the genetic transformation of *Aedes*, *Anopheles*, and *Culex* mosquitoes, the sequencing of the *Anopheles gambiae* genome, population suppression of mosquitoes using Sterile Insect Techniques (SIT) (Alphey *et al.*, 2013; Benelli *et al.*, 2016; Macias *et al.*, 2017). Sterile Insect Techniques rely on the release of large numbers of sterile males to seek, court, and mate with wild females, thereby reducing the reproductive potential of the target wild population (Alphey *et al.*, 2013). The genetic control method is a species-specific and environmentally safe as a means of controlling insect populations for reducing malaria diseases (Alphey *et al.*, 2010). Recombinant DNA methods also provide a step change in our ability to design and build highly specified, versatile, powerful genetic systems. In recent years, the incompatible insect method

(IIT) has also attracted considerable attention as an alternative to the classical SIT method (Ritchie *et al.*, 2018).

2.5.4. Chemical control

One of the key methods for managing vector populations and for minimizing the spread of human pathogens is chemical insecticides (Paul *et al.*, 2006). The most common practices among chemical control are indoor residual house-spraying (IRS), where the inside walls, the ceiling, and sometimes the outside eaves, porches, and nearby animal sheds are sprayed with a persistent insecticide (Okumu & Moore, 2011). DDT and some other insecticides (two carbamates, two organophosphates, and five pyrethroids) are commonly used for residual house-spraying in malaria control programs. The use of DDT for indoor residual spraying has reduced substantially over the past 40 years, but this insecticide is still considered valuable for malaria control, mainly because of its low cost relative to alternative insecticides. Despite the development of resistance to DDT in some populations of malaria vector *Anopheles* mosquitoes, DDT remains generally effective when used for house-spraying against most species of *Anopheles* (Tchigossou *et al.*, 2020).

For indoor residual-house spraying, the range of insecticides to be used depends on the local conditions of the vector (e.g. local vector vulnerability, vector actions, areas to be sprayed) and the resources available (cost of the insecticide and human power) (Okumu & Moore, 2011).

Okumu and Moore (2011) suggested that indoor residual-house spraying is considered appropriate and cost-effective where: a high percentage of structures in an operational area have adequate sprayable, surfaces can be well sprayed, the majority of vector population rest indoors, and the vector is susceptible to the insecticide in use.

Vector control in Ethiopia is carried out primarily by chemical measures, especially using indoor residual house spray (Gari and Lindtjorn, 2018). Extremely malarious localities are sprayed twice a year while moderately malarious localities are sprayed once a year (Taffese *et al.*, 2018). However, the occurrence of many vector-borne pathogens is increasing and vector control is becoming increasingly difficult because of several species becoming physiologically resistant to many of the conventional insecticides and due to the limited number of insecticides available for use in public health (Paul *et al.*, 2006). Research showing some cross-resistance between DDT and pyrethroids raises special concern. In Ethiopia, high and epidemiologically significant

resistance to DDT was reported in different areas (Taffese, *et al.*, 2018) though the level of *An. arabiensis* resistance to DDT was moderately low in some other localities (Balkew *et al.*, 2003).

Research showed that resistance was reported from DDT and some other insecticides against malaria vector. The increasing problems associated with resistance argue for resistance management through the use of multiple vector control methods, including non-chemical tactics.

2.5.5. Personal protection methods

2.5.5.1. Insecticide-treated mosquito nets (ITNs)

In different parts of the world, improved efforts aimed at disease prevention through ITNs and synthetic repellents for personal protection against biting mosquitoes had also been taken place (Wilson *et al.*, 2020).

The use of insecticide-treated nets (ITNs) is one of the most powerful interventions available for the effective reduction of malaria infection incidence, morbidity, and mortality (Belay and Deressa, 2008). It provides good protection from mosquitoes after people go to bed and when used widely in the community (Msangi *et al.*, 2008). However, there remains the problem of biting and disease transmission when people are sitting outside houses before going to bed.

Pyrethroid-treated bed nets are an effective means for controlling malaria vectors and preventing diseases for large-scale deployment in highly endemic areas (Okuma, 2020). However, the development of resistance may result from reliance on a single class of insecticides for bed net care. Thus, an alternative insecticide to monitor human disease vectors is urgently needed.

In Ethiopia, the use of ITNs was introduced in selected areas as one of the important malaria control measures in 1997/98 on a cost-recovery basis (Yehwalaw *et al.*, 2012). Meanwhile, in 1998 ITNs have been introduced throughout most endemic areas as a major control strategy (Ghebreyesus *et al.*, 2006). However, there are some problems such as ITNs can be uncomfortable to use in hot climates due to poor ventilation, net cost restricts for large use in many rural poor communities, regular net washing decreases the effectiveness of the treated net, and ITNs are restricted only at bed time but, most people are bitten outdoors before bedtime (Tassemedo *et al.*, 2021).

2.5.5.2. Repellents

Repellents are substances typically applied to the skin or clothing and other kinds of stuff

directly, such as arm and ankle bands or mosquito screens to deter an arthropod from fling to, landing on, or biting human and animal skin (Choochote *et al.*, 2007; Bekele and Petros, 2017). There are also spatial chemical repellents which are volatilized and prevent human-vector contact by disrupting host seeking behavior without smearing over the skin (Logan *et al.*, 2020). Many potential repellents can be considered as a 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 (Mapossa *et al.*, 2021). Mainly repellents are two types such as synthetic and natural (plant-based) repellents.

2.3.5.2.1. Synthetic repellents

Synthetic repellents are a common means of personal protection against biting arthropods. There are several kinds of synthetic repellents that have been developed so far. DEET 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 (Chen-Hussey *et al.*, 2017). However, concerns have been raised over the risk of adverse toxic effects, especially in young children and pregnant and lactating women (McGready *et al.*, 2001; Osimitz *et al.*, 2010).

In addition to DEET, other synthetic repellents are also reported as protecting humans from biting 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.*, 2001). KBR-3023 (a propidine compound) is another repellent that is found to be equally effective with DEET (Pretorius *et al.*, 2003). Repellents are suggested for people staying outdoors at night for work or leisure and those working in the agricultural state. Repellents are accessible in various forms (cream, lotion, soap, jelly, and oil) and modes of application. Repellents avoid human-biting insect contact by acting as an irritant to the insects (Blackwell *et al.*, 2004).

However, synthetic repellents are rarely used to protect communities from malaria and other vector-borne diseases. Cost and safety constraints are the main reasons for this situation (Costantini *et al.*, 2004; Osimitz *et al.*, 2010). There have also been concerns over the toxicity of DEET (McGready *et al.*, 2001). It irritates the eyes and mucous membrane when applied to the face.

2.3.5.2.2. Natural (plant-based) repellents

Plants have traditionally been used for insect control by people for many years. They have essential oils which are naturally occurring compounds responsible for an aromatic plant's distinctive odor (Sharifi-Rad *et al.*, 2017). These essential oils act as a defensive mechanism against insect attacks and their derivatives provide an alternative source of mosquito control (Akhtar *et al.*, 2010).

The repellent properties of plant essential oils to mosquitoes and other pest insects were well known before the advent of synthetic chemicals (Kalita *et al.*, 2013). Uses of traditional repellents are widespread among different cultures and very common in many communities. Recent research in different parts of the world focuses on plant-based repellents for mosquito control and other vector control (Asadollahi *et al.*, 2019).

Directly the plant parts or their extracts or their essential oils are used as insect repellents. There are over 344 plant species that have mosquitocidal properties (Ghosh *et al.*, 2011). The bioactive material of the plant is obtained by extraction of its seed, leaf, and other material of the plant. There are many reports for controlling mosquitoes by using botanical.

Essential oils extracted from citronella grass have repellency for mosquitoes. 100 µl and 0.1 ml of citronella grass essential oil showed 2.16 and 0.8 h complete protection time against *An. minimus* (Phasomkusolsil and Soonwera, 2010) and *An. dirus* (Sritabutra *et al.*, 2011). *Ocimum selloi* oil is an effective mosquito repellent that presents low toxicity, poses no mutagenic risk, and seems not to be irritating to human skin (Padilha de Paula *et al.*, 2003).

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 Kenya. Additionally, five most effective oils extracted from *Litsea cubeba*, *Melaleuca leucadendron*, *Melaleucaquinquenervia*, *Viola odorata* and *Nepeta cataria* have a protection time of 8hr at the maximum and 100% repellency against *Ae. aegypti*, *An. stephensi* and *Cx. Quinquefasciatus* (Amir and Mehlhorn, 2006).

Mosquito repellents contain some plant oils such as pennyroyal, citronella, eucalyptus, soybean, or peppermint as recognized active ingredients (Maia and Moore, 2011). In addition to these, essential oils with potential repellent have been obtained from plants in several families

containing several well-known repellent plants (Nerio *et al.*, 2010). Plants from Lamiaceae family including *Ocimum basilicum*, *Mentha* spp., *Hyptissuaveolens*, *Lavandula* spp., *Salvia* spp. and *Thymus* spp. Have high repellent effect (Dambolena *et al.*, 2016). The Myrtaceae family includes eucalyptus and tea tree (*Melaleuca* spp.) and the Poaceae includes citronella, lemongrass, and palmarosa (*Cymbopogon* spp.) are used as a repellent for mosquitoes (Trongtokit *et al.*, 2005; Maia and Moore, 2011).

In Ethiopia, the burning of dried repellent plants is one of the common practices to drive away insects and mosquitoes (Karunamoorthi *et al.*, 2009). It is usually performed by using the traditional charcoal stove (thermal expulsion) in the early evenings. Thermal expulsion on the burning of seeds and leaves of *S. molle*, *O. lamiifolium* and *P. abyssinica* significantly repelled endophagic mosquitoes (*An. arabiensis*, *An. phareonsis*, and *culicines*) (Atsebeha, 2005).

Yared (2007) evaluated the repellent property of essential oil of *Chenopodium ambrosioides*, *Laggera tomentosa*, *Eucalyptus camaldulensis*, *Cymbopogon citratus*, *Citrus sinensis* and *Ruta chalepensis* against *An. arabiensis* and *An. aegypti*. Among them, *L. tomentosa*, *E. camaldulensis* and *Cy. citratus* protected *An. arabiensis* for up two hours effectively but not for *An. aegypti*.

According to Bekele *et al.* (2014), *Aloe pirottae* is a promising mosquito larvicide while *Oreosyce africana* and *Piper capense* carry huge potentials as mosquito adulticides. *Albizia gummifera* (seeds), *Balanites aegyptica* (fruits), *Hedera helix* (leaves and fruits), *Millettia ferruginea* (seeds) and *Warburgia ugandensis* (leaves) exhibited promising larvicidal activities against *Aedes aegypti*, *Aedes africanus*, and *Culex quinquefasciatus*, respectively (Debella *et al.*, 2007).

Oils extracted from the leaves of *Otostegia integrifolia* and *Stephania abyssinica*, roots of *Echinops kebericho* and seeds of *Millettia ferruginea* had strong repellent activities against *An. arabiensis* (Jemberie *et al.*, 2016). In general, essential oil-derived compounds from the plant, with a few exceptions, can be applied to humans in a similar way to other conventional insecticides and they tend to be selective and have little or no harmful effects (Sharifi-Rad *et al.*, 2017).

3. MATERIALS AND METHODS

3.1. Description of the test plants

3.1.1. *Citrus limon* (L.) Burm.f, Rutaceae, Lommi

The *Citrus limon* (L.) Burm.f widely known as the Limon tree is a species from the Rutaceae family and native to Asia (Klimek-Szczykutowicz *et al.*, 2020). *C. limon* is a tree reaching up to 2.5–3 m in height. It has evergreen leaves and edible fruit. Flowers are bisexual with white and a purple tinge at the edges of the petals. The fruit is an elongated, oval, pointed green berry that goes yellow during ripening. Inside, the berry is filled with a juicy pulp divided into segments (Mabberley, 2004) (Appendix 4). The suitable condition for the cultivation of *C. limon* tree is sunny places. It grows on loamy, well-drained, moist soils with a wide pH range. *C. limon* is cultivated in different parts of the world including Ethiopia.

Citrus limon is a species with appreciated pharmaceutical, cosmetic and culinary (healthyfood) properties. It has anticancer (Raimondo *et al.*, 2015), antioxidant (Parhiz *et al.*, 2015), anti-inflammatory (Parhiz *et al.*, 2015), antimicrobial (Otang and Afolayan, 2016), antiparasitic (Aboelhadid *et al.*, 2016), anti-allergic (Tsujiyama *et al.*, 2013), antidiabetic (Mohanapriya *et al.*, 2013) and ant-obesity activity (Kim *et al.*, 2015). The main raw material for such biological activities is obtained from fruit extract, juice, and essential oil of fruit peels of *C. limon*. The valuable biological activity of *C. limon* is determined by its high content of phenolic compounds, predominantly flavonoids (e.g., diosmin, hesperidin, limocitrin) and phenolic acids (e.g., ferulic, synapic, p-hydroxybenzoic acids). The essential oil is rich in bioactive monoterpenoids such as D-limonene, β -pinene, and γ -terpinene.

Essential oil of *C. limon* has excellent repellent properties against *Anopheles stephensi* compared with the synthetic repellent of DEET (Oshaghi *et al.*, 2003). The fruit peel of citrus limon was used as insect repellent (Karunamoorthi, 2012) In Ethiopia, there is no report for evaluating repellent property of *C. limon* essential oil against malaria vectors.

3.1.2. *Kleinia odora* (Forssk.), (Asteraceae), *Kleinia spp.*

Kleinia odora is native to Ethiopia, Kenya, Oman, Saudi Arabia, Somalia, Tanzania, Uganda, Yemen, and Djibouti. It has thick, branched, cylindrical stems which are decumbent or pendulous on cliffs. Leaves reduced to subulate scales up to 3 cm long, these arising from wart-

like outgrowths on the stem (Cicuzza *et al.*, 2017) (Appendix 4). The habitats of *Kleinia odora* are Acacia bushland, semi-desert scrub, often on rocky hillsides; may be thicket-forming, and possibly thrives in over-grazed areas. The plant is located in the Raya-azebo district of Tigray, the Kalu district of the Amhara region, and the Harla and Dengego villages of the Eastern region of Ethiopia. The vernacular name of this plant is duea in Kalu, bierir in Raya-azebo, luko in the Eastern region (Harla and Dengego village).

People in the Eastern region of Ethiopia use *K. odora* oily extract as a treatment for nerve cases (Belayneh and Bussa, 2014). People in Raya-Azebo used *K. odora* as a repellent against mosquitoes and houseflies by fumigation in their houses traditionally (Gidey, 2018). However, the repellent activity of its essential oil has not been evaluated against malaria vectors in Ethiopia.

3.1.3. *Securidaca longepedunculata* (Fresen.), (Polygalaceae), Violet tree

Securidaca longepedunculata belongs to the family Polygalaceae. It is a medium-sized violet tree indigenous to Ethiopia. It is known by the vernacular names in Amharic: ‘Temene’, ‘Etse-Menahe’, ‘Etse-Menabele’. The common English names for the plant are Violet Tree, Rhodes’s Violet and Wild Vesteria. It is found widespread in tropical Africa from Eritrea and Ethiopia to South Africa, occurring in semi-arid savannah and deciduous lowland woodland. In Ethiopia, it grows in Dry and Moist *Bereha* and *Kolla* agro-climatic zones, common at Benishangul-Gumuz and Gambella. It also occurs in Tigray, Gondar, Gojam, Wollega, Showa, Illubabor, and Gamo Gofa in various kinds of woodlands and bushlands (Azene, 2007).

It is a semi-deciduous shrub or small tree growing up to a height of 2-6 meters, with a characteristic pale smooth bark with slender branches to an open crown, sometimes with drooping branchlets. Leaves are variable in size and shape, alternate, often in clusters or crowded on dwarf spine-tipped branchlets. Leaves have fine hairs but mature leaves lose the fine hairs. Flowers in short bunches are pink or purple with a sweet scent (Junaidu *et al.*, 2014). The bark of young twigs is yellow-green, becoming stringy and pale; rough grey mature bark flakes to show yellow below; deep fissures when old. It is resistant to bush fires and is frost-sensitive (Azene, 2007) (Appendix 4).

The violet tree, *S. longepedunculata*, known as the king of traditional medicinal plants in South Africa, is used for almost every conceivable ailment (Kadiri *et al.*, 2013). To treat chest complaints, inflammation, abortion, tuberculosis, infertility, heart, kidney, lung, venereal diseases, and constipation, root and bark are taken orally, powdered, or as an infusion (Orwa *et al.*, 2009). The leaves or roots ground with water and salt is used against snake bites and cough (Sanusi *et al.*, 2014). Emmanuel *et al.* (2020) reported that *S. longepedunculata* have excellent repellent activity against *An. gambiae s.l.* and *Cu. quinquefasciatus* mosquitoes. However, the repellent property of *S. longepedunculata* essential oil is not evaluated against mosquito vector in Ethiopia.

3.1.4. *Warburgia ugandensis* (Sprague.), (Canellaceae), pepper bark tree

Warburgia ugandensis belongs to the family Canellaceae. It is native to Africa including Ethiopia. It is a spreading evergreen tree 4.5-30 m tall with a diameter up to 70 cm. Its bark is scaly, and has pale green, brown, or slashes pink color. The leaves have dotted glands. Its flowers are solitary and bisexual; its kidney-shaped bracts only cover the young buds. The petals are dotted with glands. The tree has a berry fruit, which at first is green and later turns purplish with a leathery glandular skin (W AFC, 2002) (Appendix 4).

Warburgia ugandensis is a plant with immense medicinal values with restricted distribution in tropical Africa (Muller *et al.*, 2015). It occurs in lowland rainforest, upland dry evergreen forest and its relicts in secondary bushland and grassland; also on termitaria in swamp forest. In Ethiopia, the plant is located in Gimbi in Wellega and Dollo Menna in Bale district. The vernacular name of the plant is zogdom in Amharic and mukabiftu (biftu) in Afan-oromo.

It is a highly valued medicinal plant in traditional medicine with a broad spectrum of antimicrobial activity whose parts especially the leaves and stem bark have for long been used in the treatment and management of many diseases and health conditions such as stomachache, cough, toothache, fever, malaria, oral thrush, measles and diarrhea in African communities where the plant occurs (Wamalwa *et al.*, 2006).

Sylvia *et al.* (2020) reported that extracts from *W. ugandensis* are effective for controlling maize insect pests, *Prostephanus truncates*. But, the essential oil repellency property of *W. ugandensis* is not evaluated against mosquitoes in Ethiopia.

3.1.5. *Rosmarinus officinalis* (L.), Lamiaceae, Rosemary

Rosmarinus officinalis commonly known as rosemary is a plant species that belongs to the family Lamiaceae and is native to the Mediterranean region. Now a day, it can be cultivated all over the world including in Ethiopia. It is a small perennial and aromatic plant with green leaves that produce a distinctive fragrance. It is shrub-shaped with branches full of leaves and can grow up to two meters tall. *R. officinalis* is a versatile plant that may be used as a spice in cooking, a natural preservative in the food industry, and an ornamental and medicinal plant (González-Trujano *et al.*, 2007; Perez-Fons *et al.*, 2010; Raskovic *et al.*, 2014) (Appendix 4).

Numerous phytochemicals can be extracted from *R. officinalis* root, stem, leaves, flower, fruit and bark for pharmacological activity as essential oil or extract. Caffeic acid, carnosic acid, chlorogenic acid, monomeric acid, oleanolic acid, rosmarinic acid, ursolic acid, alpha-pinene, camphor, carnosol, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin are among the most commonly reported phytochemical from essential oil and extract (Martínez *et al.*, 2009; Einbond *et al.*, 2012; Borges *et al.*, 2019).

Rosmarinus officinalis essential oil possesses antimicrobial, antioxidant, anti-carcinogenic, cognition-improving, and glucose-reducing qualities, making it an excellent natural animal feed additive (Debersac *et al.*, 2001; Fu *et al.*, 2007).

According to Govindarajan *et al.* (2011) report, *R. officinalis* essential oil has effective repellent property against *An. subpictus* species. However, there is no report for evaluating the repellent property of essential oil of *R. officinalis* against *An. arabiensis* in Ethiopia.

3.1.6. *Lavandula angustifolia* (Miller.), Lamiaceae, lavender

Lavandula angustifolia is a perennial shrub that belongs to the mint family Lamiaceae. It is native to southern Europe and the Mediterranean area and is commercially cultivated in France, Spain, Portugal, Hungary, the UK, Bulgaria, Australia, China, and the USA (Shawl and kumar, 2000). It is cultivated for its aromatic inflorescence from which the essential oil is isolated. The essential oil of this species is commonly used in aromatherapy and massage. It has major clinical benefits on the central nervous system. The essential oil is also known for its excellent aroma and is extensively used in the perfumery, flavor, and cosmetic industries.

Chu and Kemper (2001) also stated that Lavender extracts have traditionally been prescribed to treat infertility, infection, anxiety, and fever, and have been used as antidepressants, antispasmodics, anti-flatulent agents, antiemetic remedies, and diuretics.

According to Amer and Mehlhorn (2006) report, *L. angustifolia* essential oil has good repellent properties against *An. stephensi*. The essential oil of *L. angustifolia* has the highest repellency against adult *Hyalomma marginatum rufipes*, tick (Mkolo and Magano, 2007). However, there is no report about the repellent property of *L. angustifolia* against mosquitoes in Ethiopia.

3.2. Collection of plant materials

The plants were collected from different parts of the country. The collected plants were identified at Plant Biology and Biodiversity Management Department of Addis Ababa University. The plant part used for extraction of essential oil of the six plants and their locations are listed in the Table 1.

Table 1 Summary of plants were used in the laboratory for extraction of essential oil by Hydro distillation and their localities

S.No.	Plant species	English name	Plant parts used for extraction	Locality of collection
1	<i>Citrus limon</i> (L.) Burm.f	Lemon rut	Peels	Boru
2	<i>Kleinia odora</i> (Forssk.)		Stem	Dengego around Dire Dewa
3	<i>Securidaca longepedunculata</i> (Fresen.)	Violet tree	Root bark	Assosa
4	<i>Warburgia ugandensis</i> (Sprague.)	Pepper bark tree	Leaves	Bale-Goba
5	<i>Rosmarinus officinalis</i> (L.)	Rosemary	Leaves	Addis Ababa
6	<i>Lavandula angustifolia</i> (Miller.)	Lavender	Leaves	Addis Ababa

3.3. Essential oils extraction

Six plant essential oils were chosen for this study. All were extracted by Clevenger hydro distillation (Appendix 5) in the Traditional and Modern Medicine Research Directorate

laboratory of the Ethiopian Public Health Institute (EPHI). In this process, the parts of the plants were cut into small pieces and placed into a distillation flask with much water. The distillation chamber was heated at about 120⁰c and allowed to boil until the distillation was completed.

The distillate was collected in a separating funnel in which the aqueous portion separated from the volatile oil. The water layer was slowly drawn off until only the oil layer was remaining. The oil was collected in a cylinder at about 4⁰ c until it was tested for mosquito repellency.

3.4. Test mosquitoes under laboratory

An. arabiensis mosquitoes reared at the Infectious Directorate of EPHI were used for the study. The strain of *An. arabiensis* was originated from around Adama. Mosquitoes were reared using WHO standard procedures (WHO, 2013). The mosquitoes were maintained at 25 ± 2⁰C and 80 ± 10% relative humidity. Stock populations of adult mosquitoes were provided with 10% sugar solution. Mosquitoes were contained during testing using a cage with a solid bottom and top, screen or netting on the back, a clear acrylic sheet (for viewing) on the right and left sides, and a fabric sleeve for access on the front.

Female mosquitoes were collected from a stock population cage in which both sexes have been maintained to allow mating to occur. They were host-seeking, of uniform age, preferably 5-7 days post-emergence. The experiment was conducted following guidelines for efficacy testing of mosquito repellents for human skin (WHO, 2009).

3.5. Dose-response tests of repellents

Serial dilutions (0,3%, 0.6%, 1.25%, 2.5% and 5%) of repellent were made with ethanol and tested to identify effective dose ranges. Each volunteer uses incremental doses on the test forearm so that each volunteer used five successive applications of increasing doses. A single test comprises continuous use of the same mosquitoes by the same volunteer and was completed in one day. Replicate tests repeat this process using different batches of mosquitoes over three days. Three replicates were conducted per volunteer.

One ml of ethanol used in the preparation of the test repellent was applied evenly using a pipette to approximately 600 cm² of the forearm skin between the wrist and elbow and allowed to dry for 2 min. Before insertion of the arm into the cage containing 50 female mosquitoes, the hand was protected by gloves made of a material through which the mosquito cannot bite. The first step

was inserting the forearm applied with ethanol into the cage and counting the number of mosquitoes that were landed on and/or commenced to probe the skin during 30 seconds.

During testing, the volunteer was not allowed to move their arm. The test was allowed to proceed if the biting rate was ≥ 10 landings and/or probing in the 30 seconds.

The control forearm was carefully withdrawn and this arm was then treated with the lowest dose of repellent in 1 ml ethanol and allowed to dry. The treated arm was placed in the cage for another 30 seconds and observed for mosquito landings and/or probe.

This procedure was applied for each additional incremental repellent dose. Successive tests were carried out one after the other without delay. Testing was not proceeding when the mosquito landing and/or probing rate on the exposed forearm were <10 females within 30 seconds. This procedure was used consistently throughout the experiment. The number of landings and/or probing was recorded by the trained volunteers (Appendix 6).

3.6. Estimation of complete protection time

One ml of 20% ethanoic DEET solution was compared with the one ml of 5% candidate repellent on the other arm. In both cases, the treatment was applied on an approximately 600 cm^2 area of the forearm skin between the wrist and elbow.

Two mosquito cages (size: 35-40 cm per side) each containing 100 non-blood-fed females were normally used. One cage was designed for testing the candidate repellent and the other for the positive control (ethanoic DEET). Initially, the readiness of mosquitoes to land and/or probe was assessed by inserting an untreated (ethanol-treated) arm into a cage for 30 seconds until 10 landing/probing were counted. The procedure was repeated with the other arm in the second cage.

Before testing commences, 1 ml of the candidate repellent prepared in ethanol solution was applied to one arm and 1ml of the DEET standard solution was applied to the other arm. After 30 minutes, the repellent treated arm was inserted into the appropriate cage and exposed for 3 minutes to determine to land and/or probing activity.

Next, the DEET applied arm was exposed to determine to land and/or probing activity. This procedure was repeated at 30-minute intervals and was used consistently throughout the experiment. The occurrence of one landing and/or probing in a 3-minute test interval concludes

the test for that repellent dose. Complete protection time is calculated as the number of minutes elapsed between the time of repellent application and the first mosquito landing and/or probing.

The experiment was conducted following the guidelines for the efficacy testing of mosquito repellents for human skin (WHO, 2009).

3.7. Ointment Formulation

10% and 5% (W/V) ointment were formulated by combining 0.5ml of essential oil in 5g petroleum jelly and 0.25ml of essential oil in 5g petroleum jelly respectively for testing of chemicals of the plant for acute dermal irritation. 10% (W/W) SLS ointment was formulated by combining 0.5g of sodium lauryl sulphate 99% with 5g of petroleum jelly as a positive control.

3.8. Experimental animals

Healthy, adult Swiss albino mice (weighting 27.9 –36.2g, 10-12 weeks of age, female) and healthy, adult guinea-pigs (weighing 0.8-1.2kg, 6-7 weeks of age, male) were obtained from Ethiopian Public Health Institute, Addis Ababa, Ethiopia. Animals were housed in a standard cage in a ventilated room under room temperature of $25 \pm 2^{\circ}\text{C}$ with a 12hr light/dark cycle. Animals were acclimatized for a minimum of 5 days to the laboratory conditions prior to experimentation and were fed with standard food pellets and water ad libitum. Maintenance of animals and experimentation was performed following the “Principles of Laboratory Animal Care” (NIH publication number 85-23 revised in 1985).

3.9. Skin irritation test

The experiment was conducted according to the protocol for the testing of chemicals for acute dermal irritation/corrosion (OECD, 2002). Nine adult guinea-pigs were used for the experiment. Twenty-four hours before the experiment, furs from the backs of all guinea-pig were clipped on at different sites. Half a gram of 10% ointment formulation was evenly and gently applied in a test site while untreated skin areas served as a control. The test sites were then examined critically at 1hr after removing the test material and at 24hrs, 48 hrs, 72 hrs, 7th and 15th day for the dermal reaction using Draize scoring criteria (OECD, 2002; Kamkaen *et al.*, 2007).

3.10. Acute toxicity study

All animals were deprived of food for 3-4 hours prior to commencing the experiment. Thirty female mice were randomly divided into six groups each having five female mice.

Doses of 300mg/kg, 600mg/kg, 1200mg/kg, 2400mg/kg, 4800mg/kg body weight candidate EO were administered orally from group 1 to 5 female mice respectively (OECD, 2008). The last group (group-6) of 5 female mice as a negative control group was administered with 5ml of 2% tween 80. Each mouse was observed critically during the first four hrs periodically for the first 24hrs, 48hrs, 72hrs, 7 days, and once a day for 14 days. During this period the activities related to motor-muscle coordination and the central and autonomic nervous systems were analyzed (Appendix 7).

3.11. Data Analysis

Repellency percentage of essential oils was evaluated in both the dose response studies at each time interval in the evaluation of protection time following Yap *et al.* (1998).

$$\% \text{ Repellency} = \frac{C - T}{C} \times 100$$

Where; C is the average of the landing/probing on the two untreated arms (the diluents – applied test arm before repellent treatment and the other arm at the end of the experiment and; T is number of mosquitoes landing/biting on treated arms.

Data was analyzed using computer software for probit-plane regression analysis by SPSS version 26. From which, the median effective dosage (ED50) and ED95 of the oils were analysed. Effectiveness of the test oils was determined by comparing the 95% confidence intervals of the ED50 and ED95 values. The percentage repellency among the oils was compared using one-way ANOVA, and the means were separated using Tukey's honestly significant difference (HSD) test at P=0.05.

3.12. Ethical Consideration

An experiment on repellents was undertaken following the applicable national ethical regulations. I received the ethical clearance certificate from Addis Ababa University ethical review committee. Each test experiment was conducted based on the voluntariness of the participants. WHO guidelines for the development of an informed consent form were applied.

4. RESULTS

4.1. Dose-response studies of the essential oils

This study evaluated the repellent effects of essential oils of six plants against laboratory-reared *An. arabiensis* at different concentrations (0.3%, 0.6%, 1.25%, 2.5%, and 5%) at dose-response studies.

Table 2 shows the ED₅₀ and ED₉₅ values of the volatile oils of candidate repellent plant species against *An. arabiensis* in dose-response studies based on probit analysis. *W. ugandensis* showed the lowest ED₅₀ (0.019%; 95% CI=0.000- 0.105) and ED₉₅ (0.410%; 95% CI=0.003 - 0.946) values, thus showing highest repellent activity among the candidate plants. *S. longepedunculata* was the weakest repellent plant from the candidate plant with ED₅₀ (0.103%; 95%CI= 0.014-0.219) and ED₉₅ (3.253%; 95% CI=1.836-12.871). Based on the 95% confidence interval, there were no significant differences among all the plant oils at both studied doses (ED₅₀ & ED₉₅).

Table 2. The ED₅₀ and ED₉₅ values of essential oils of the six test repellent plants against *An. arabiensis* in dose-response studies.

Plant species	ED50(95% CI)	ED95(95%CI)	X ² (DF=3)	P value
<i>S. longepedunculata</i>	0.103 (0.014-0.219)	3.253 (1.836-12.871)	3.354	0.243
<i>C. limon</i>	0.045 (0.001-0.139)	1.749 (1.016-7.295)	1.869	0.969
<i>R. affinalis</i>	0.047 (0.001-0.144)	2.201(1.234-11.160)	2.260	0.751
<i>L. angustifolia</i>	0.057 (0.002-0.158)	2.380 (1.342-11.180)	2.435	0.622
<i>K. odora</i>	0.034 (0.000-0.121)	0.740 (0.393-1.988)	2.159	0.592
<i>W. ugandensis</i>	0.019 (0.000-0.105)	0.410 (0.003-0.946)	0.698	0.354

4.2. Duration of repellency of the essential oils

This study investigated that the duration of protection of essential oils of the six repellent plant species (*C. limon*, *K. odora*, *L. angustifolia*, *R. affinalis*, *S. longepedunculata* and *W.*

ugandensis) against *An. arabiensis* under laboratory condition at 5% concentrations in relation to the standard synthetic repellent (DEET).

The duration of protection at 5% concentration of essential oils against *An. arabiensis* is summarized in Figure 1. All the essential oils from the six plants gave very high protection (> 89%) immediately after application until about 2.5 hours (150 min). Whereas, the four plant essential oils (*S. longipedunculata*, *C. limon*, *R. officinalis* and *L. angustifolia*) protected the biting of *An. arabiensis* up to 3 hours (180 min) with mean protection of 82.68% - 95.65%. After four and half hours, their protections declined from 55.45% - 70.19% down to 23.19% - 65.97% after six hours of post-application. *S. longipedunculata* produced the weakest protection among the six plant oils, giving 66.26% protection at four hours and down to 23.19% at six-hour post-application. DEET, the standard commercial repellent, gave much longer repellency (80.97% - 100%) for as long as six hours of the test period against *An. arabiensis*. Moreover, the effectiveness of repellency decreased as the protection time increased (Figure 1, Table 3).

Statistically, the mean percentages of repellency among the six plant oils and DEET at 5% concentration were significant ($p < 0.05$) from 120-360 minutes after application. However, it was insignificant until 90 minutes of application ($P > 0.05$).

Table 3. Mean percentage protection \pm SE of the six plant essential oils and the standard DEET against *An. arabiensis* at 5% concentration.

Treatments	Hours post Application (Minutes)											
	30	60	90	120	150	180	210	240	270	300	330	360
Sl	100 \pm 00a	100 \pm 00a	100 \pm 00a	95.96 \pm 2.02 b	91.5 \pm 2.42b	84.8 \pm 3.10b	71.7 \pm 5.18d	66.3 \pm 5.15d	55.5 \pm 7.02 d	52.4 \pm 1.20e	43.9 \pm 10.41e	23.2 \pm 5.25c
Cl	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	95.7 \pm 2.20a	85.9 \pm 0.84bc	82.16 \pm 2.3b	77.16 \pm 1.48c	75.09 \pm 1.03c	70.08 \pm 4.44bc	65.97 \pm 1.83ab
Ro	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	93.7 \pm 0.37b	85.2 \pm 1.50b	71.9 \pm 1.68d	71.03 \pm 2.4c	67.42 \pm 4.6c	56.56 \pm 2.97e	53.40 \pm 4.32de	48.89 \pm 1.11b
La	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	89.6 \pm 1.95b	82.7 \pm 2.88b	76.8 \pm 1.03cd	73.2 \pm 1.03c	70.19 \pm 1.93c	61.00 \pm 3.77d	59.18 \pm 6.53cd	53.06 \pm 4.81b
Ko	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 0 0a	88.0 \pm 2.87b	80.0 \pm 3.62b	67.16 \pm 5.24c	61.00 \pm 3.77d	52.79 \pm 7.19de	50.97 \pm 2.80b
Wu	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 0 0a	100 \pm 00a	100 \pm 00a	88.86 \pm 1.47b	81.96 \pm 1.37b	80.27 \pm 5.69ab	76.67 \pm 1.67a
DEET	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	89.20 \pm 2.74a	80.98 \pm 3.37a
Sig. (p)	NS	NS	NS	*	*	*	*	*	*	*	*	*

Keynotes- Mean percentage protection of the six plant essential oils and the standard DEET was compared using one-way ANOVA, and the means were separated using Tukey's honestly significant difference (HSD) test at P=0.05. NS=Non significant; *= Significant at (p<0.05) level.

4.3. Skin irritation assessment

In this study, no irritation signs or skin edema and erythema were observed on guinea pigs after treatment with both 5% and 10% ointment of *W. ugandensis* EO (Table 4). The treated skin was

intact with no inflammation and erythema as the normal guinea-pigs (negative control). Edema and erythema score was “0” in each guinea-pig at any time of the observation after removing the test material. This demonstrated that the skin Primary Irritation Index score was zero (Table 4).

Table 4. Average score of edema and erythema for 5% and 10% *W. ugandensis* essential oils and 10% sodium lauryl sulfate after removing the test formulation.

Concentration	Reaction	Average skin irritation score per time interval					
		1hr	24hrs	48hrs	72hrs	7days	15days
5%	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
10%	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
Negative control	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
Positive control	Edema	0	0	0	1	1	1
	Erythema	0	0	0	1	1	1

4.4. Acute oral toxicity study

4.4.1. Behavioral pattern and LD₅₀

The results of an acute oral toxicity study showed that the essential oil of *W. ugandensis* appeared to be safe up to the dose of 2400 mg/kg. Testing parameters like vomiting, diarrhea, drowsiness, urination, skin color, fur erection, and food and water intake were assessed (Table 5). Urination was observed at a dose of 4800 mg/kg whereas diarrhea, drowsiness and erection of fur were observed at a dose of 2400 and 4800 mg/kg. Death was observed at a dose of 4800 mg/kg. Nevertheless, other groups did not show any sign of toxicity. Generally, the study revealed that there is a sign of toxicity at 2400mg/kg and 4800mg/kg for some of the settled parameters such as diarrhea, drowsiness, erection of fur and urination. All mice died at 4800

mg/kg. Therefore, LD₅₀ of the essential oil might be considered to be not greater than 2400 mg/kg.

Table 5. General appearance and behavioral observations of acute toxicity study for control and treated groups.

Observation	Dose of extracts in mg/kg					
	Control	300	600	1200	2400	4800
Food intake	Normal	Normal	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal	Normal	Normal
Vomiting	Not seen	Not seen	Not seen	Normal	Normal	Normal
Diarrhea	Not seen	Not seen	Not seen	Not seen	Present	Present
Urination	Normal	Normal	Normal	Normal	Normal	Present
Breathing	Normal	Normal	Normal	Normal	Normal	Normal
Skin color	Normal	Normal	Normal	Normal	Normal	Normal
Drowsiness	Not seen	Not seen	Not seen	Not seen	Present	Present
Hypersensitivity	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen
Erection of fur	Not seen	Not seen	Not seen	Not seen	Present	Present
Sedation	Not seen	Not seen	Not seen	Not seen	Not seen	Not seen
Death	Alive	Alive	Alive	Alive	Alive	Present(5)

Keynotes: n=5, “present” means at least 1 out of the 5 mice showed the symptom, “not seen/normal” means no mice showed the symptom.

4.4.2. Body weight

Weekly body weight of mice that were treated for different concentrations of *W. ugandensis* essential oils and 20% Tween 80 was weighed on the initial day, 7th, and 14th days of the six groups as displayed in Table 6. On the initial day, all the treated groups didn’t show any statistically significant difference in the body weight compared with the control group. All the

groups except those treated under 4800 mg/Kg body weight showed a very slight and non-significant weight loss on the 7th day but reversed it on the 14th day. However, the group of mice treated at 4800 mg/Kg showed a significant difference to the controlled group and all other groups on the 7th and 14th days. At this level, mortality of all the mice within the group was observed (Table 6).

Table 6. The effect of *W. ugandensis* essential oils on the body weight of mice at different days

Group	Doses(mg/kg)	Weight(gm)		
		Initial day	7 th day	14 th day
1	20% tween80	31.72±3.42 ^a	31.18±3.19 ^a	31.02±2.74 ^a
2	300	31.44±2.60 ^a	30.52±2.01 ^a	31.52±2.12 ^a
3	600	31.38±1.36 ^a	30.42±1.93 ^a	31.08±2.59 ^a
4	1200	31.16±2.13 ^a	30.20±2.32 ^a	31.30±2.83 ^a
5	2400	31.68±2.94 ^a	28.72±2.33 ^a	32.20±1.10 ^a
6	4800	31.94±1.50 ^a	0.00±0.00 ^b	0.00±0.00 ^b
Total		31.55±2.24	25.17±11.65	26.19±12.08

Keynotes: Values are expressed as Mean ± SD (n=5) one-way ANOVA followed by Tukey's multiple comparison tests (P<0.05), "Means" that do not share a superscript letter are significantly different (only column-wise).

5. DISCUSSION

The present study showed that essential oils of six plants had acceptable percentage of biting protection against *An. arabiensis*. Dose-response result indicated non-significant difference among the six plant essential oils at both ED₅₀ and ED₉₅ values. This implied that the average dosage of all essential oils for their 50% and 95% repellent efficacy did not have any statistical difference even though, the oils were extracted from different plant species. All the plant species tested might have common metabolite having mosquito repellent efficiency that made them to have non-significance difference in the dosage. As oppose to this, Mulugeta Wano (2006) observed a significant difference between six essential oils in ED₅₀ and ED₉₅ against *Aedes aegypti* and *An. arabiensis*. In agreement, Yared (2007) showed non-significant difference in the effective median dose of six plants' essential oils against *An. arabiensis*. Likewise, non-significant difference was observed among all of the six essential oils at ED₉₅ against *Aedes aegypti* (Yared, 2007). However, a significant difference between some essential oils in ED₅₀ and ED₉₅ against *Aedes aegypti* and *An. arabiensis*, respectively were reported (Yared, 2007). The presence of slight variation in secondary metabolites composition is expected not only between different plants species but also with in individuals of the same species due to environmental variations from which the plants are grown on. This could be the reason that showed slight difference with the present study result and Yared's (2007) result.

The repellency duration of all essential oils was non-significant with DEET for two and half hours with 100% protection under similar conditions. But, it continues only on essential oils of *K. odora* and *W. ugandensis* for up to three hours and this extends to four hours only for *W. ugandensis*. This indicated that all plant species under examination had essential oils with great insect repellent ability. However, their durability difference might be associated with the volatility difference among the oils. The presence of volatile chemicals in plants being evaporated before or after extraction was reported by Otsyina *et al.* (2000). *W. ugandensis* showed an excellent repellent efficacy with an average repellency of 93.98% within six hours which is nearly comparable to the normal synthetic repellent DEET which had an average of 97.51% repellency effect. This plant might produce powerful bioactive secondary metabolites that made it to have better repellent value than the others species examined. The report of Opiyo *et al.* (2015 and Opiyo (2020) also showed the presence of effective insect repellent essential oils

in the leaves of *W. ugandensis*. This plant species had also strong mosquito larvicidal effect as Asfaw Debella (2007) reported.

K. odora, the average protection time was 83.34% with three hours complete protection time that made it second next to *W. ugandensis*. In line with this, the essential oil of *K. squarrosa* which is under the same genera as *K. odora* provided three hours of complete protection time against *An. gambiae* with a similar concentration (5%) (Mulugeta Guta *et al.*, 2017). The occurrence of many essential oils (AL-Taweel *et al.*, 2004) along with their disturbing odor (Mulugeta Guta *et al.*, 2017) might have contributed for the repellency of *K. odora* against mosquitoes.

Concerning *C. limon* had 65.97% - 100% repellent ability in average of 87.67% within six hours and two and half hours complete protection. In agreement with this, the repellent activity of essential oils from *C. limon* against *An. stephensi* was also reported over human and animal skins (Oshaghi *et al.*, 2003). Eventhough, the average repellency effect put this plant at second rank, 100% complete protection time was 30 minutes less than that of *K. odora*. This might be related to the presence of fast volatile essential oils in *C. limon* that would made measurable effect on the complete protection time. Hojjati *et al.*, (2017), Kaskoos (2019) and Paw *et al.* (2020) reported the presence of different *C. limon* essential oils constituent at different concentration and Khanikor *et al.* (2021) reported the volatile nature of about 85–99% of these compounds. In comparison to this volatility nature, the oil of *C. sinensis* (the plant species which shares same genus to *C. limon*) oil against *An. arabiensis* and *Aedes aegypti* did not give more than one hour of significant protection (> 80%) (Yared, 2007). The peel essential oil of *Citrus hystrix*, which is relative species to *Citrus limon*, is also reported to possess a repellent effect against *Ad. aegypti* and *An. minimus* (Nararak *et al.*, 2017).

L. angustifolia placed 4th in its repellent efficacy with a mean of 80.49% and 120 minutes of 100% protection time. In parallel to this, essential oils of the plant showed a 100% repellent effect for 150 minutes against *Aedes aegypti* at concentrations of both 10% and 20% and going reduced by the increased exposure periods as Paulraj (2021) reported which is 30 minutes more than *An. Arabiensis*. This might be directly related to the concentration of the oils under test as Yared (2007) and Pratiwi and Purwati (2021) described. It could also be the higher resistance ability of *An. arabiensis* for the repellent over *Aedes aegypti*.

In addition, Baranitharan *et al.* (2021) reported the presence of different essential oils from *L. angustifolia* that had larvicidal and ovicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. Quinquedasciatus*. From these, it is possible to recommend that *L. angustifolia* could have both repellent and insecticidal applications.

R. officinalis recorded 5th in its 5% essential oils repellent durability with 79.01% average repellency and two hours of complete protection. But, it reduced to 48.89% after six hours of exposure and this is observed in most plant essential oils since they have volatile nature as reported by Khanikor *et al.* (2021). Its average repellent effect is nearly equal to *L. angustifolia* (80.49%) and 120 minutes of complete repellency. This might be the availability of similar essential oil constituents in the two species since; both are belonging to the same family (*Lamiaceae*). In agreement with this study result, Gillij *et al.* (2008) and Pratiwi and Purwati (2021) reported repellent effect of *R. officinalis* essential oils against *Aedes aegypti* with decrease in efficiency as time increased. This could be associated with the loss of volatile constituents and the odor (fragrance) as the authors described.

Securdaca longipedunculata had 90 minutes complete repellency power with an average of 73.76% although; the repellency effect was reduced to 23.19% after six hours of application. This made *S. longipedunculata* to have the least repellency power in this study. In agreement with this, Bossou *et al.* (2013) reported the lower repellent and toxic effect of *S. longipedunculata* for *Anopheles gambiae* which belongs under the same genus of *An. arabiensis*. This poor repellent effect of *S. longipedunculata* might be related to the nature and types of secondary metabolites produced. The report of Nebie *et al.* (2004) and Bossou *et al.* (2013) indicated the presence of only one major essential oil constituent in *S. longipedunculata* which is characterized by having volatile nature as Jayasekara *et al.* (2002).

Dermal irritation data are crucial parts of the safety assessment of herbal medications for authentication (Ema *et al.*, 2012) stated. Thus, a skin irritation test of *W. ugandensis* essential oil formulation is essential toxicity screening that provides a fundamental characterization of the potential hazards to the skin. The prepared ointment was evaluated for its skin irritant effect, where no erythema or edema was observed with PII equal to 0 for both 5% and 10% (W/V) indicating that the prepared herbal formulation is safe for topical use. This implied that no immunological response is observed for superficial application of *W. ugandensis* essential oils

since skin irritation is a physiological reaction due to local inflammation (Clough *et al.*, 2002). Even, using more concentrations of this essential oil could not show skin irritation symptoms if it is smeared over human skin since human skin is about 40% more resistant than animal skin as Basketter *et al.* (2004) reported. Thus, essential oils of *W. ugandensis* could be the better mosquito repellents by replacing chemical repellents if attention is due considered.

An acute toxicity study is needed to establish a safer dose range to manage the clinical signs and symptoms of the drugs (Saleem *et al.*, 2017). Accordingly, an oral toxicity test was carried out for different concentrations of *W. ugandensis* essential oils on mice. The result revealed the presence of some behavioral changes like diarrhea, drowsiness, and erection of fur for the doses of 2400 mg/Kg and 4800 mg/Kg body weight. Likewise, an increase in urination and total deaths were observed on the dose of 4800 mg/Kg. This indicated that the LD₅₀ of *W. ugandensis* leaves essential oil was found to be not greater than 2400 mg/kg and thus based on the scale of Loomis and Hayes (1996) classified it as slightly toxic for internal use.

In line with its slight toxicity, cytotoxic sesquiterpenes called muzigadials in *W. ugandensis* metabolites was observed (Olila *et al.*, 2001). In contrast, Karani *et al.* (2013) and Ngugi (2020) showed the absence of any mortality or behavioral pattern change in BALB/c mice except restored overcrowding and inactivity until the doses of 5000 mg/Kg body weight of essential oils of this plant and this dose is scaled as practically non-toxic (Loomis and Hayes, 1996). Oloya *et al.* (2022) also reported the absence of any mortality and change in behavior in adult female Wistar albino rats by 2000 mg/Kg stem bark essential oils of *W. ugandensis*. The toxicity variation might be appeared due to differences in the plant parts where essential oils were extracted. These previous authors had extracted the essential oils from the stem bark of the plant but, it was from the leaves in the current study. Studies also showed the presence of more essential oils from leaves than stems (Silva *et al.*, 2011; Barbouchi *et al.*, 2021). Thus, the presence of slight toxicity in the current study might be the presence of toxic chemical constituents in the leaves which are not found in the stems.

Mbwambo *et al.* (2009) also demonstrated that ethanolic leaf extracts of *W. ugandensis* exhibited cytotoxic activity against brine shrimp. This might also be happened due to variation in type and amount of essential oil constituents within the same plant species as a result of environmental factors as Lahkimi *et al.* (2020) described. Even though slight internal toxicity was observed, the

result of this study showed the absence of dermal toxicity of this plant's essential oil (Table 4).

The body weight changes serve as a sensitive indicator of the general health status of animals (Hilaly *et al.*, 2004). Very slight and non-significance decrease in weight of all experimental groups except those treated at 4800 mg/Kg body weight on the 7th day indicated inhibitory effects of the drug in the metabolic activities or its interference with cells functioning as Gathumbi *et al.* (2000) described. Further reversion to the initial body weight on the 14th day might be associated with the development of immune response in the first few days after treatment and rehearsing to the normal cellular functioning of the mice. A similar phenomenon was also observed in rats treated at 2812.15 mg/kg body weight essential oil of *P. africana* in one study (Karani *et al.*, 2013). In parallel, other studies using restraint stress reported rats' weight loss and hypophagy until a few days after the stress had ended (Harris *et al.*, 1998). In contrast, Karani *et al.* (2013) reported the presence of non-significant but progressive weight gain of rats treated at 2812.15 mg/kg body weight essential oil of *W. ugandensis* stem bark. This might be variation in chemical constituents of different plant parts (Silva *et al.*, 2011; Barbouchi *et al.*, 2021). It could also be environmental dependency of chemicals for their concentration Lakhimi *et al.* (2020). However, the overall mortality of the mice treated at 4800mg/Kg body weight starting from the 7th day after treatment could indicate the inability of the animals to regain their normal metabolic activities due to overdosage. This would be a good pointer to the slight toxicity of *W. ugandensis* essential oils extracted from leaves.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This investigation showed that the essential oils of six plants provided a good percentage of biting protection against *An. arabiensis*. The Environmental protection Agency (EPA) has set 2 hours minimum activity requirement for a plant-derived repellent to gain registration. Accordingly, essential oils from all the test plants with the exception of *S. longependunculata* gave a promising result against *An. arabiensis* with more than 2 hours repellent efficacy at 5% concentration. Essential oils of these local plants have promising protection against mosquito biting and could be natural alternatives to synthetic repellents. Although high repellent activity was recorded on essential oils of *W. ugandensis*, slight acute toxicity was observed. But it is safe for topical use. The toxicity of essential oil is dependent on different factors like environmental factors on which the plant is growing on, the plant part under study, or maturity stages since, they determine the concentration, composition and bioactivity of secondary metabolites.

6.2. Recommendations

Based on the findings of this study, the following recommendations are forwarded;

- ✓ Valuable and active constituents of essential oils from these plant species for their repellency effect should be understood, screened, and characterized.
- ✓ In addition, possibilities to stabilize the essential oils for their prolonged repellent effect should be developed by changing the type of solvents used.
- ✓ To confirm the non-toxic nature of the essential oils from these plants, different variables such as; plant part, environmental factor where the plants are grown and growth stage should be looked into.
- ✓ Moreover, further investigations should be done using and the need to plan for future pre-clinical and clinical studies of the medicinal plants should be considered.
- ✓ Optimization of repellent formulation
- ✓ Studies on other malaria vectors

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8. APPENDICES

Appendix 1 Mean percentage protection \pm SE of the six plant essential oils and the standard

DEET against *An. arabiensis* at 5% concentration.

	Hours post Application (Minutes)											
	30	60	90	120	150	180	210	240	270	300	330	360
Sl	100 \pm 00a	10 \pm 00a	100 \pm 00a	95.96 \pm 2.02 b	91.5 \pm 2.42 b	84.8 \pm 3.10 b	71.7 \pm 5.18 d	66.3 \pm 5.15 d	55.5 \pm 7.02 d	52.4 \pm 1.20 e	43.9 \pm 10.41 e	23.2 \pm 5.25c
Cl	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00 a	95.7 \pm 2.20a	85.9 \pm 0.84bc	82.16 \pm 2.38 b	77.16 \pm 1.48c	75.09 \pm 1.03c	70.08 \pm 4.44bc	65.97 \pm 1.83ab
Ro	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00a	93.7 \pm 0.37 b	85.2 \pm 1.50b	71.88 \pm 1.68 d	71.03 \pm 2.41 bc	67.42 \pm 4.61c	56.56 \pm 2.97e	53.40 \pm 4.32de	48.89 \pm 1.11b
La	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00a	89.6 \pm 1.95b	82.7 \pm 2.88b	76.83 \pm 1.03cd	73.25 \pm 1.03 bc	70.19 \pm 1.93c	61.00 \pm 3.77d	59.18 \pm 6.53cd	53.06 \pm 4.81b
Ko	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	88.03 \pm 2.87b	80.09 \pm 3.62 b	67.16 \pm 5.24c	61.00 \pm 3.77d	52.79 \pm 7.19de	50.97 \pm 2.80b
Wu	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	100 \pm 00a	88.86 \pm 1.47b	81.96 \pm 1.37b	80.27 \pm 5.69ab	76.67 \pm 1.67a
DE ET	100 \pm 00a	10 \pm 00a	100 \pm 00a	100 \pm 00 ^a	100 \pm 00 a	100 \pm 00 a	100 \pm 00 a	100 \pm 00 a	100 \pm 0a	100 \pm 00 a	89.20 \pm 2.74a	80.98 \pm 3.37a
Sig. (p)	NS	NS	NS	*	*	*	*	*	*	*	*	*

NS=Non significant; *= significant at (p<0.05) level.

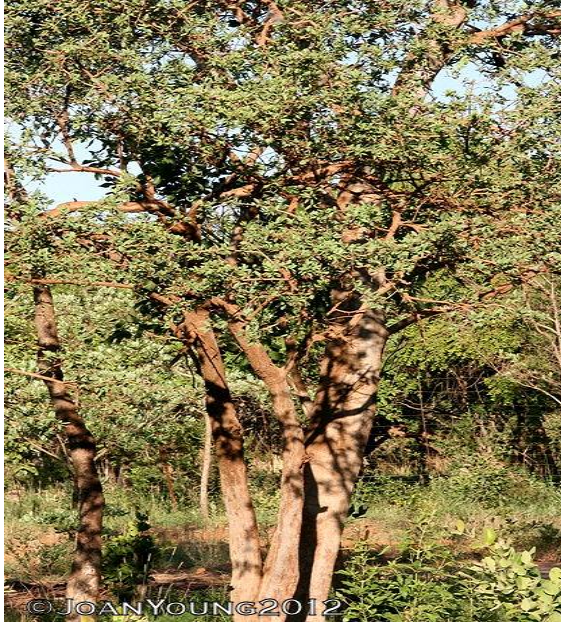
Appendix 2 Average score of edema and erythema for 5% and 10% *W. ugandensis* essential oils and 10% SLS after removing the test formulation

Concentration	Reaction	Average skin irritation score per time interval					
		1hr	24hrs	48hrs	72hrs	7days	15days
5%	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
10%	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
Negative control	Edema	0	0	0	0	0	0
	Erythema	0	0	0	0	0	0
Positive control	Edema	0	0	0	1	1	1
	Erythema	0	0	0	1	1	1

Appendix 3 The effect of *W. ugandensis* essential oils on the body weight of mice at different days

Group	Doses(mg/kg)	Weight(gm)		
		Initial day	7 th day	14 th day
1	20% tween80	31.72±3.42 ^a	31.18±3.19 ^a	31.02±2.74 ^a
2	300	31.44±2.60 ^a	30.52±2.01 ^a	31.52±2.12 ^a
3	600	31.38±1.36 ^a	30.42±1.93 ^a	31.08±2.59 ^a
4	1200	31.16±2.13 ^a	30.20±2.32 ^a	31.30±2.83 ^a
5	2400	31.68±2.94 ^a	28.72±2.33 ^a	32.20±1.10 ^a
6	4800	31.94±1.50 ^a	0.00±0.00 ^b	0.00±0.00 ^b
Total		31.55±2.24	25.17±11.65	26.19±12.08

Key notes: Values are expressed as Mean ± SD (n=5) one-way ANOVA followed by Tukey's multiple comparison tests (P<0.05), "Means" that do not share a superscript letter are significantly different (only column wise).



Securidaca longepedunculata

(Source: <https://natureswow2.blogspot.com>)



Citrus limon

(Source: <https://en.wiktionary.org>)



Kleinia odora

(Source: <https://www.google.com>)



Rosmarinus officinalis

(Source: <https://www.gardenersworld.com>)



Lavandula angustifolia

(Source: <https://www.provenwinners.com>)



Warburgia ugandensis

(Source: <https://www.flickr.com>)

Appendix 4 Images of the test plant



Appendix 5 Essential oil extraction material (Hydrodistillation)





Appendix 6 Human volunteers exposing their arm in experiment



Appendix 7 Image for acute oral toxicity studies on mice



Appendix 8 Images for skin irritations studies on guinea-pigs