Addis Ababa University

College of Natural and Computational Sciences

Department of Zoological Sciences

Larvicidal effect of some plant extracts against *Anopheles arabiensis* Patton (Diptera: Culicidae) under laboratory condition in Ethiopia

By

Araya Eukubay

Thesis Submitted to the Graduate School of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Zoological Sciences (Insect Sciences)

June, 2019

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DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been presented for degrees in any other university and all sources of material used for the thesis have been duly acknowledged.

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ABBREVIATIONS/ACRONYMS

IRS	Indoor Residual Spraying
ITN	Insecticide treated net
LC_{50}	Lethal Concentration that kill 50% of the exposed larvae
LC_{90}	Lethal Concentration that kill 90% of the exposed larvae
LLIN	Long-lasting insecticide treated nets

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ABSTRACT

Despite the availability of control interventions, malaria remains as a major public health problem in Ethiopia. The emergence and spread of malaria vector resistance to insecticide is limiting the efficacy of current control interventions. Natural plant products have a vital role to resolve this problem. In this study larvicidal activities of *Artemisia annua* L. (Asteraceae), *Calpurnia aurea* (Aiton) (Fabaceae) *Clausena anisata* (Wild) Hook. F.ex. Benth (Rutaceae), *Datura stramonium* L. (Solanaceae) and *Ricinus communis* L. (Euphorbiaceae) ethanol and methanol leaf extracts were evaluated against *Anopheles arabiensis* larvae under laboratory conditions. Larvicidal bioassay was conducted in three replicates per treatment each with a total of 25 3rd instar *An. arabiensis* larvae at different concentrations ranging from 50-300 ppm. The larval mortality were observed after 24 hours and percentage mortality were recorded. LC_{50} and LC_{90} were also determined using log probit analysis. Furthermore, methanol extract of *C. aurea* was subjected to bioassay guided column chromatographic fractionation and seven fraction were tested for their larvicidal activities. Qualitative secondary metabolite screening was also conducted for the crude extract of test plants. All the plant crude extracts were found to have a potential larvicidal activity against 3rd instar *An. arabiensis* larvae. The highest larvicidal activity was observed in methanol crude extract of *C. aurea* with 100% larval mortality at 300 ppm and lowest LC_{50} of 84.85 ppm and LC_{90} of 192.29 ppm. Ethanol crude extract of *R. communis* also achieved 93.33% larval mortality at 300 ppm with the LC_{50} and LC_{90} of 134.52 ppm and 304.67 ppm, respectively. The lowest larvicidal activity was observed in methanol extracts of *A. annua* with 68% larval mortality at 300 ppm. Fraction F1 and F3 leaf methanol extracts of *C. aurea* showed an excellent larvicidal activities with mortality of 100% and 98.66 % at 250 ppm and LC_{50} of 62.51 and 82.33 ppm, respectively after 24 hours exposures while F5-F6 showed no larvicidal activity in all test concentrations. Besides, methanol extract of *C. aurea* showed the presence of alkaloids, flavonoids, phenol, terpenoids, tannin and saponin. The present study revealed that column chromatographic F1 and F3 of methanol extract of *C. aurea* exerted a remarkable larvicidal activity against *An. arabiensis* and thus these can be used for botanical mosquito insecticide development. Further study need to be conducted to identify the active ingredients in the column chromatographic fractions of F1-F3 of methanol extracts of *C. aurea* and their mode of actions.

Key words: *Anopheles arabiensis*, larvicide, plant extract, column chromatography

1. INTRODUCTION

1.1. Background

Despite the availability of control interventions malaria remains as a global public health problem. Globally, about 3.4 billion people in 91 countries are at risk of infection (http:// www. who. int/gho/malaria/en/). An estimated of 219 million malaria cases occurred worldwide in 2017 with 92% of them occurring in WHO African region (WHO, 2018). Malaria is caused by protozoan parasite of genus *plasmodium* (Cox, 2010). In Ethiopia, in spite of the decline in morbidity and mortality, malaria case incidence is still high and remains as a major public health problem (Taffese *et al.*, 2018). Human malaria is transmitted by female mosquitoes of genus *Anopheles*, and not all anophelines are reported as vectors of malaria. In Africa *Anopheles gambiae*, *An. coluzzii*, *An. arabiensis* and *An. funestus* are the major malaria vectors. The first three species are member of the *An. gambiae* complexes which also includes *An. bwambae*, *An. melas*, *An. merus*, *An. quadriannulatus* and *An. amharicus* (Coetzee *et al*., 2013). *An. arabiensis*, a member of the *An. gambiae* complex, is the primary malaria vector in Ethiopia, with *An. funestus*, *An. pharoensis*, and *An. nili* considered as secondary vectors (PMI, 2016). Recently non-African malaria vector- *An. stephensi* is also reported from the Ethiopian Somalia region (Carter *et al*., 2018).

Currently, five species of *Plasmodium* parasites are known to infect humans. These are *Plasmodium falciparum, P.vivax, P. ovale, P. malariae* and *P. knowlesi*. *P. falciparum* is the most severe form and can lead to coma and death within few days (Howard, 2010). It is widespread in Africa and is responsible for 99% of estimated malaria cases in Sub-Saharan Africa in 2016 (WHO, 2017), while *P.vivax, P. ovale*, and *P. malariae* infections are less common and geographically restricted (Nkumama *et al*., 2017) . In Ethiopia, *P. falciparum* and *P. vivax* are the major malaria parasites. According to the Ethiopia National Malaria Indicator Survey (EMIS) *P. falciparum* accounts for about 63.7% and *P. vivax* constitute 36.3% of cases (MIS, 2015).

Vector control is a crucial prevention tool to mitigate mosquito borne diseases. Historically, it has resulted in a successful eradication of malaria in different part of the world (Raghavendra *et al*., 2011). Vector control strategies have evolved considerably since Müller's discovery of the insecticidal properties of DDT in the 1939 which targets the adult female mosquitoes (Sougoufara *et al*., 2017). During the Global Malaria Eradication Programme, indoor residual spraying (IRS)

of insecticides (in combination with environmental management, improved housing and treatment) has produced profound changes in malaria burden in a range of settings, including its elimination in the USA, Europe, parts of the Soviet Union, Israel, Lebanon, Syria, Japan, and Taiwan. In Africa IRS was largely overlooked during the GMEP (Oxborough, 2016). In 2012, a total of 88 countries, including 40 countries in the African Region, implemented IRS for malaria control.

Currently, insecticide treated nets (ITNs)/Long-lasting insecticidal nets /LLINs) and IRS are two of the most effective malaria prevention strategies recommended for use in sub-Saharan Africa. Moreover, other interventions such as effective case management and malaria rapid diagnostic tests (RDTs) are parts of the malaria control, prevention and elimination strategies (Woyessa *et al*., 2014). Both ITNs and IRS were being scaled up throughout sub-Saharan Africa during the last 10 years (Gimnig *et al*., 2016). The use of ITNs/ LLINs effectively prevent malaria transmission by forming the physical barrier between the infected mosquitoes and man as well as repelling and killing of the vectors (Raghavendra *et al*., 2011). The massive scale-up of insecticide-treated nets resulted in more than 50% of people in malaria endemic areas in sub-Saharan Africa sleeping under nets in 2016. Of 663 million clinical malaria cases averted in sub-Saharan Africa since 2001, 78% were averted due to the use of ITNs and IRS (Kleinschmidt *et al*., 2018).

Although larval control of malaria vector *Anopheles* mosquitoes has become neglected, it is a proven preventive method that deserves renewed consideration for malaria control programs in the twenty-first century (Walker & Lynch, 2007). Recent field evaluations under various eco-epidemiological conditions in Africa revealed that hand-applied larviciding reduced transmission by 70-90% where the majority of aquatic mosquito larval habitats were defined and aquatic surface areas not too extensive (Fillinger & Lindsay, 2011). The main advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and, unlike adult mosquitoes, cannot easily avoid control measures (Walker & Lynch, 2007).

Malaria vector control in Ethiopia accounts about half a century and currently it relies on LLINs and IRS. IRS in Ethiopia was initiated in 1959 with the global malaria eradication campaign. Blanket spraying with DDT continued until the late 1970s in almost all affected areas. In the early 1980s, the eradication program was transformed into a control program with IRS as the major intervention. Blanket spraying was replaced by selective application. The use of only DDT

continued until the early 1990s when time-limited replacement with malathion was considered in selected areas where vector populations resistant to DDT were encountered (WHO African Region, 2007). The use of DDT for IRS was banned by 2010 due to spreading of DDT resistant malaria vectors and replaced with deltamethrin (Messenger *et al*., 2017). In 2011 pyrethroid-based IRS was replaced by carbamate, due to the high level of resistance in malaria vector populations. Currently, carbamate insecticides are in use for IRS (Zerihun *et al*., 2018). LLINs have been also used starting from end of August 2005 as malaria control interventions in Ethiopia (Animut *et al*., 2008). According to EMIS about 64% of households dwell in malarious areas (<2000masl) of the country used LLINs in 2015 (MIS, 2015).

The emergence and spread of resistances of malaria vectors to the four insecticide classes currently used in ITNs and IRS threatens malaria prevention efforts. Of the 73 malaria endemic countries that provided monitoring data to WHO for 2010 onwards, 60 reported resistance to at least one insecticide in one malaria vector from one collection site, and 50 reported resistance to two or more insecticide classes. Resistance to pyrethroids – the only class currently used in ITNs is the most commonly reported (WHO, 2016a). In Ethiopia malaria vector resistance to DDT, deltamethrin, perimethrin, lamdacyhlothrin, alphacypermethrin, malathion, propoxure and moderate resistance of bendicarbes have been reported from different parts of the country (Abate & Haddis, 2010; Balkew *et al*., 2010; Balkew *et al*., 2012; Massebo *et al*., 2013a; Fettene *et al*., 2013; Abraham *et al*., 2017). This will hinder to meet the objectives of malaria global technical strategies (MGTS) 2016-2030 which aimed in malaria elimination by 2030 (WHO, 2016b). The development of other eco-friendly and cost-effective vector control method is essential to resolve the problem of resistance and reduce environmental pollution.

Natural plant products derived from various botanical sources have provided numerous beneficial uses ranging from pharmaceuticals to insecticides (Raghavendra *et al*., 2011).Various studies have focused on the use of natural products, especially plant-derived essential oils, as suitable bioactive agents against larvae of *An. gambiae* s.s. and other mosquito species (Kweka *et al*., 2016). Plant based products are preferable because they possess a wide range of bioactive phytochemicals that are selective, biodegradable, and have minor or no adverse effects on non-target organisms and the environment, making them potentially appropriate for use in integrated vector management programs. Published reports indicated that essential oils and extract of local plants such as

Cymbopogon citratus, Croton macrostachyus, Jatropha curcas, Eucalyptus globules, Mentha spicata, Nigella sativa, Ocimum lamiifolium, Thymus vulgaris, Aloe pirottae, Piper capense and *Phytolacca dodecandra* have a promising larvicidal, adulticidal and repellent activities against *An. arabiensis* in Ethiopia (Karunamoorthi & Ilango, 2010; Tomas *et al*., 2011; Massebo *et al*, 2013b; Bekele *et al*., 2014; Berhe, 2015; Zeleke *et al*., 2017). Other plants such as *Clausena anisata* (Wild) Hook. F. ex. Benth (Rutaceae), *Ricinus communis* Lnn. (Euphorbiaceae), *Datura stramonium* L. (Solanaceae) and *Artemisia annua* L. (Asteraceae) are reported to have larvicidal activity on *Culex salinarius, C. quinquefasciatus, An. stephensi, An. gambiae, An. arabiensis, An. supictus , Aedes albopictus and Ae. aegypti* in other countries other than Ethiopia while no study was conducted on mosquitocidal activities of *Calpurnia aurea* (Aiton) Benth (Fabaceae) elsewhere (Tandon *et al*., 2010; Govindarajan, 2010; Mandal, 2010; Taha *et al*., 2011; Ghosh *et al*., 2012; Govindaraja & Sivakumar, 2013; Ollengo *et al*., 2016; Sharma *et al*., 2016; Dahchar *et al*., 2016; Ghebriel & Adugna, 2017; Aouinty *et al*., 2018). Thus, this study was focused on the evaluation of larvicidal activity of these plants against *An. arabiensis* under laboratory conditions in Ethiopia.

1.2. Objectives

1.2.1. General objective

To investigate the larvicidal activities of plant extracts against *An. arabiensis* under laboratory condition

1.2.2. Specific objectives

- ✓ To evaluate larvicidal efficacy of crude methanol and ethanol leaf extracts of *Calpurnia aurea*, *Clausena anisata*, *Ricinus communis*, *Datura stramonium* and *Artemisia annua* against late third instar larvae of *An. arabiensis*
- ✓ To determine the LC_{50} and LC_{90} values of crude leaf extracts of *Calpurnia aurea*, *Datura stramonium* and *Ricinus communis* against late third instar *An. arabiensis* larvae
- ✓ To evaluate the larvicidal activities of column chromatographic fractions of methanol leaf extract of *Calpurnia aurea* against late third instar *An. arabiensis* larvae
- ✓ To identify the phytochemical constituents of crude leaf extract of the candidate plants

2. LITERATURE REVIEW

2.1. Malaria

Malaria is an ancient disease that has plagued humans throughout history. The Greek physician Hippocrates described malaria in his writings during the 400s BC. Throughout history and even today outbreaks of malaria have often been associated with warfare, migrations, and other societal disruptions (Cowman *et al*., 2016). It is caused by parasites belonging to the genus plasmodium and is endemic in more than 104 countries in tropical and subtropical countries (Kar, *et al*., 2014). Four species of human malaria parasites –*Plasmodium falciparum*, *P. malariae*, *P.ovale* and *P.vivax* have been identified although the fifth non-human primate parasite- *P.knwlesi* is found to infect humans (Su, 2010). *P. falciparum* is widespread in Africa while *P. vivax, P. ovale,* and *P. malariae* infections are less common and geographically restricted (Nkumama *et al*., 2017). In India, *P. vivax*, has been the primary pathogen responsible for malaria, even though P. *falciparum* cases are on the rise in recent times (Mandal *et al*., 2011). Globally, an estimated 3.4 billion people in 91 countries and territories are at risk of being infected with malaria and developing disease and 1.1 billion are at high risk (>1 in 1000 chance of getting malaria in a year) (http://www.who.int/gho/malaria/en/). According to (WHO, 2017) in 2016, an estimated 216 million cases of malaria occurred worldwide compared with 237 million cases in 2010. Compared with 2015, 5 million more malaria cases were estimated to have occurred globally in 2016 with 90% of the cases occurred in the WHO African Region followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean. An estimated of 445000 malaria deaths with about 91% deaths in the WHO African Region occurred globally in the same year. Reports of (PAHO/WHO, 2018) indicated that an increase in number of malaria cases was observed in 2015 and 2016, and most recently in 2017 in the Region of the Americas.

2.1.1. Malaria in Africa

Despite the significant decline in malaria cases and deaths being reported globally where the incidence rate of malaria worldwide declined steadily from 76 to 63 cases per 1000 population at risk in 2010 to 2016 and from 256 to 206 cases per 1000 population at risk in 2010 to 2016 in the WHO African Region, malaria remains a major public health problem in Sub-Sahara Africa (Kateera *et al*., 2016; WHO, 2017). The epidemiology of malaria varies geographically depending on the local malaria transmission intensity or endemicity class. Although the exact numbers may

be uncertain, it is estimated that 395 000 deaths from malaria were occurred in Africa in 2015 (Nkumama *et al*., 2017). In 2016 about 407000 (approximately 91%) of malaria deaths occurred in WHO African region (WHO, 2017). Malaria causes 10 % of all deaths of children under the age of 5 years in sub-Saharan which is equivalent to one child in sub-Saharan Africa dying of malaria every 2 min.

Both the parasite and the mosquito vectors are affected by climate. Mosquitoes are unable to survive in low humidity and their breeding grounds are expanded by rainfall. *Plasmodium* parasites are also affected by temperature where their development slows as the temperature drops and stops at high temperatures, which is the reason why parasites can be found in temperate areas (Roberts & Matthews, 2016).

Malaria in pregnancy has a devastating effect on the health of mothers and their babies, and is an important cause of maternal and infant mortality and morbidity. The greatest effect of malaria in pregnancy is concentrated in sub-Saharan Africa and is associated with *P. falciparum* infection. However, pregnant women are also at risk of *P. vivax* malaria. Although its burden seems to be lower than that of *P. falciparum*, *P. vivax* malaria is still associated with harmful consequences for maternal and infant health (Menéndez *et al*., 2015).

P. vivax and *P. falciparum* are the primary causes of malaria in humans and until recent years, the majority of malaria research and funding has been focused on the prevention, treatment, and control of *P. falciparum.* Both parasite species expose approximately 2.5 billion people to risk of infection (Howes *et al*., 2016). *P. falciparum* is the most prevalent malaria parasite in sub-Saharan Africa, accounting for 99% of estimated malaria cases in 2016 (WHO, 2017). Even though, the overall *P. vivax* prevalence in Africa remains low, the parasite is considered to be present in the Horn of Africa, and recently its presence is also reported from West Africa where it is absent before (Poirier *et al*., 2016) . Its unique biological and epidemiological characteristics pose challenges to control strategies that have been principally targeted against *P. falciparum*. Unlike *P. falciparum*, *P. vivax* infections have typically low blood-stage parasitemia with gametocytes emerging before illness manifests, and dormant liver stages causing relapses (Howes *et al*., 2016).

Malaria causes direct and indirect economic losses in Africa. Direct economic loss is due to expenses related to treatment or prevention where it is already costing the continent's economy USD 12 billion a year in direct losses and indirect economic loss is because of absentiseem and

decrease in productivity. In Ghana, for example, malaria treatment accounts for about 34% of the incomes of poor households (Tabbabi, 2018). A high malaria burden is likely to increase labor turnover resulting in increased hiring and training costs and reduced profitability for enterprises in the formal sector, like plantation farms and reduce improved agricultural practices of farmers as well. Furthermore, a high malaria incidence within a particular area may undermine tourism opportunities, deter otherwise profitable foreign and domestic investment, and prevent the use of land or other natural resources. On the other hand, some agricultural practices and development interventions are known to facilitate the spread of malaria, exacerbating its impacts (Asenso-Okyere *et al*., 2011).

2.1.2. Malaria in Ethiopia

Malaria remains as a challenging problem in Ethiopia despite the progress in reducing the burden of malaria and other major communicable diseases over the last two decades. It is still among the ten top leading causes of morbidity and mortality in children under-5 years (Deribew *et al*., 2017). More than 54 million populations living in about 75% of landscapes of the countries are at risk of contracting the disease. The area especially below 2000 m above sea level is fertile low land area and suitable for agriculture. This area is also good for malaria parasite and vectors adaptations as well. *P. falciparum* and *P. vivax* are the two species commonly known to cause malaria in Ethiopia accounting for 60% and 40% proportion, respectively (Alelign & Dejene, 2016).

Malaria transmission has bimodal type of transmission where it peaks between September to December and April to May, coinciding with the major harvesting seasons. This has serious consequences for Ethiopia's subsistence economy and for the nation in general. Major epidemics occur every five to eight years with focal epidemics as the commonest form (Ayele *et al*., 2012). Malaria transmission in Ethiopia varies from place to place as the distribution is determined by altitudes which could in turn affect transmission distribution through effects on the temperature. Risk of malaria is highest in the western lowlands of Oromia, Amhara, Tigray and almost the entire regions of Gambella and Benishangul Gumuz regions (Alelign & Dejene, 2016). True explosive epidemic malaria was recorded at exceptionally high altitude (around 2500m) from Ethiopia (Negash *et al.*, 2005). This could be due to the warming of climate that favors both the parasite and vectors of the disease in highland areas of the countries (Lyon *et al*., 2017).

Malaria poses a significant economic burden on rural households and individuals both through out-of-pocket payment and person-days lost (Deressa *et al*., 2007). For instance study conducted

in South-central Ethiopia revealed that the median cost of malaria per episode to the household was USD 5.06. The direct cost accounted for 39%, while the indirect counterpart accounted for 61% (Alemayehu *et al*., 2017).

2.2. Malaria vectors

Human malaria parasites are transmitted by the mosquitoes of the genus *Anopheles*. It is believed that about 465 species of Anopheline have been formally recognized and there are also more than 50 unnamed members of species complexes. Approximately 70 of these species have the capacity to transmit human malaria parasites and 41 are considered here to be dominant vector species/species complexes (DVS), capable of transmitting malaria at a level of major concern to public health (Manguin, 2013).

2.2.1. Malaria vectors in Africa

Dealing with the distribution of malaria vector mosquitoes, especially those belonging to species complexes that contain vector species, is important for strategic planning of malaria control programmes (Coetzee *et al*., 2000). *Anopheles* species from the *gambiae* complex -*Anopheles arabiensis, An. gambiae, An. coluzzii*, *An. melas* and *An. merus* and from the *funestus* subgroup *An. funestus* are considered as the most important vectors transmitting both *P. falciparum* and *P. vivax* parasites to humans (Wiebe *et al*., 2017). *An. gambiae* s.s. Giles and *An. arabiensis* Patton, sibling species in the *An. gambiae* Giles complex, are found together through much of their home ranges with *An. arabiensis* tending to be more frequent where it is hot and dry. In East African context *An. arabiensis* tends to be more zoophilic whereas *An. gambiae* s.s. is anthropophilic, though in Mali and West Africa both species seem to be, for all practical purposes, completely anthropophilic (Edill *et al*., 2002). *An*. *merus* is exclusively confined to the Eastern coast stretching from South Africa to the horn of Africa. Existing reports revealed that this species is mainly zoophilic. Analysis of blood meal sources from anopheline mosquitoes collected along the coast of Kenya showed that *An*. *merus* predominantly fed on humans (Kipya *et al*., 2013). *An. melas* is a salt-tolerant species mostly found in coastal environments, the mangrove ecosystem where its larvae develop in brackish water (Attolou *et al*., 2016).

2.2.2. Malaria vector in Ethiopia

It is believed that there are about 45 species of Anopheles mosquitoes in Ethiopia (Animut, 2016). *Anopheles arabiensis*, a member of *An. gambiae* species complex is the principal malaria vector

and widely distributed in the country (Yohannes *et al*., 2005; Animut *et al*., 2012; Masseebo *et al*., 2013c; Kenea *et al*. 2016; Taye *et al*., 2016: Abraham *et al*., 2017). *An. pharonesis*, *An. funestus* and *An. nilli* are other vectors which occur in Ethiopia (Animut *et al*., 2012; Taye *et al*., 2016). *An. demeilloni* positive for *P. falciparum* is also reported from south Ethiopia (Taye *et al*., 2016). *An. amharicus* previously known as *An. quadriannulatus* sp. B, is zoophagic and has not been incriminated as malaria vector (Massebo *et al*., 2015). Moreover, *Anopheles stephensi*, a malaria vector typically found in the Middle East, the Indian subcontinent, and China, but recently found in Djibouti was also detected in the Ethiopian Somalia region (Carter *et al*., 2018).The density of Anopheles mosquitoes varies with altitudinal transects (Animut *et al*., 2013; Taye *et al*., 2016).

2.3. Biology and Bionomics of *Anopheles arabiensis*

Anopheles arabiensis belongs to the *An. gambiae* species complex, and is one of the most important vectors of malaria in sub-Saharan Africa (Coetzee *et al*., 2000). Ecological niche modelling revealed *An. arabiensis* to be a climate generalist in the sense that it can occur in most of Africa's contemporary environmental range (Drake & Beier, 2014). *An. arabiensis* and *An. gambiae* mosquito are considered as r-strategist. They are able to colonize suitable aquatic habitats within a few days after they are created as predators may be less prevalent in a small temporary habitats, and larval food may be more abundant than it is in long-lasting habitats though some study indicated that stable habitats are more productive than temporary unstable habitats (Minakawa *et al*., 2005; Munga *et al*., 2013). *An. arabiensis* prefers generally small, temporary, sunlit, clear and shallow fresh water pools and is able to utilize a greater variety of locations than *An. gambiae*, including slow flowing, partially shaded streams and a variety of large and small natural and man-made habitats. It has been found in turbid waters, on occasion, in brackish habitats and irrigated rice fields (Sinka *et al*., 2010). As small and sunlit habitats have higher water temperatures, mosquito larval–pupal developmental time may be shortened if the warmer habitat produces more algal food and larval mortality due to desiccation or poor nutrition may be reduced until water temperatures rise above 30°C (Minakawa *et al*., 2005).

Physicochemical parameters survey in various mosquito breeding sites has revealed that the quality of water ranges from fresh to highly pollute. Fresh mosquito breeding habitats are clean waters with little organic matter dissolved while highly polluted mosquito breeding habitats are sites with high levels of salinity, ammonium ions and organic pollution.

Variation in physicochemical parameters of mosquito breeding sites at various levels has some influence on mosquito vector oviposition, survival and spatial distribution (Emidi *et al*., 2017). Research conducted in Sudan indicated that some populations of *An. arabiensis* larval forms are adapted to breed in polluted urban habitats. These changing of breeding site may contradict the efficacy of larval control intervention (Azrag & Mohammed, 2018). *Culex quinquefasciatus* and *An. arabiensis* larvae were found to ere positively associated with dissolved oxygen. The optimum dissolved oxygen might have contribution for survival and breeding of *Anopheles* larvae. Vegetation is also important predictor for *Anopheles* and *Culex* larvae presence and abundance. The presence of vegetation could help the larvae to hide themselves from their predators (Dejenie *et al.*, 2011). However, the occurrence of anopheline was found to decrease with increasing water depth and percentage of tall riparian vegetation (Elleby & Feltelius, 2014). Besides emergent plants and/or canopy cover reduces the amount of sunlight reaching the aquatic habitats, thereby reducing water temperature and thus reduce microbial growth upon which mosquito larvae feed (Mereta *et al*., 2013). *An. pharoensis* larvae is found abundantly in herbaceous swamps in the major drainage systems and irrigation areas preferring the permanent lakeshore vegetated water body for breeding. *An. squamosus* larvae is also sympatric with *An. pharoensis* in such habitats (Kenea *et al*., 2011).

Like all mosquitoes the life-cycle of *An. arabiensis* passes through four distinct stages: the egg, larva, pupa and adult. The time taken for the various stages to develop depends on temperature and nutritional factors, with development more rapid at higher temperatures. After mating and blood-feeding Anopheles lays some 50–200 small brown or blackish boat-shaped eggs on the water surface. Anopheles eggs cannot withstand desiccation and in tropical countries hatch within 2–3 days, but in colder temperate climates hatching may not occur until after about 2–3 weeks. Once the eggs are hatched emerging larvae floats below and parallel to water surface where they can breathe air. The larval stages passes through four developmental stages (I-IV instars) within an average of 8–10 days at normal tropical water temperatures. The 4^{th} instar larvae pupates in to the non-feeding comma shaped pupa and undergoes a major transformation, from living in water to becoming a flying adult mosquito. It remains floating at the water surface, but when disturbed they swim vigorously down to the bottom with characteristic jerky movements. The pupal period lasts 2–3 days in tropical countries but sometimes as long as 1–2 weeks in cooler climates, after which the adult mosquito emerges. Once the adult emerges from the pupa it undergoes mating and

search for blood meal. Normally the female takes the first blood-meal only after mating, but sometimes the first blood meal is taken by young virgin females. The first batch of eggs develops after one or two blood meals (depending on the species) while successive batches usually require only one blood-meal. The female usually mates only once because sufficient sperm are received from a single mating for all subsequent egg batches (Service, 2012).

2.4. Behavior of *Anopheles arabiensis*

Understanding of behavior of vector species – their ecology and related microclimate are crucial to set an appropriate control intervention (Lelisa *et al*., 2017). Different species could have different behavior such as resting habit, oviposition site selection, egg deposition, feeding habit, host preference, feeding time, specific part of host on which they feed. Behavioral variation could also exist within species in different areas depending on the environmental conditions (Paaijmans & Thomas, 2011;Bashar *et al*., 2012; Braack *et al*., 2015; Lelisa *et al*., 2017).

2.4.1. Biting activity

Identifying biting cycle of mosquito is an important to make decision for malaria control intervention (Elleby & Feltelius, 2014). Even though all anopheline vector species predominantly feed at night, extensive applications of insecticides as control measures leads to dramatically variation in biting activity of *An. arabiensis* across Africa (Fornadel *et al*., 2010; Gatton *et al*., 2013). For instance, In Zambia, *An. arabiensis* biting occurs throughout the night, with peak activity starting before midnight at approximately 10:00 pm. Peak biting after midnight has been also observed in Senegal, Chad, and Kenya. However, in Mozambique and Tanzania, high activity levels were seen as early as 9:00 pm (Fornadel *et al*., 2010). In Ethiopia, a study made by Yohannes and Boelee (2012) revealed that most of the biting activity of *An. arabiensis* occurred before 22:00 hours and thus before the time when people usually go to bed. On the other hand, high bed time (22:00-5:00) indoor biting activity of parous *An. arabiensis* and outdoor human biting activities during the early part of the night were observed in South-central Ethiopia (Kenea *et al.*, 2016). In South western Ethiopia, the highest peak indoor and outdoor biting activity for *An. gambiae* s.l., *An. custani* and *An. pharonsis* is between 18:00 and 21: 00 hours. Biting activity for *An. gambiae* s.l. both indoor and outdoor also increased between 03:00 and 06:00 hours (Taye *et al*., 2016). 18:00-19:00 hour indoor peak biting and 19:00-20:00 hour outdoor peak biting of *An.arabiensis*,

An. pharonsis and *An. custani* have been described from Central part of Ethiopia (Kibret *et al*., 2010).

2.4.2. Feeding and resting behavior

Many female *Anopheles* mosquitoes bite humans to obtain a blood meal, and a few feed on humans in preference to animals. Mosquitoes are attracted to hosts by various stimuli emanating from their breath or sweat, such as carbon dioxide, lactic acid, octenol, body odours and warmth. Some species feed more or less indiscriminately at any time of the day or night. After having their blood meal, mosquitoes seek resting places in which to shelter until their meal is digested and their ovaries are matured. Adults of *An. gambiae* s.l. are primarily indoor-feeding (endophagic) as opposed to outdoor-feeding (exophagic) mosquitoes. Few mosquitoes entirely feed on humans (anthropophagic) or animals (zoophagic), or some of them feed both on animal and human depending on the availability (Animute, 2016).

Mosquito can be classified as endophily- those which prefer to rest indoor and exophily-those which tend to rest outdoor (Gatton *et al*., 2013) and thus, species varies in their preference of resting places. *An. arabiensis* which occupies over 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.

Extensive vector control interventions are now causing vectors to change their feeding behavior. For example the study of Sy *et al*. (2018) showed that more aggressive outdoors biting of *An. arabiensis* in areas where IRS is applied in Senegal. *An. gambiae* s.l. mosquitoes currently seek hosts in outdoor venues as much as indoors in the Punta Europa region of Bioko Island which contrasts with an earlier pre-intervention observation of exclusive endophagy of *An. gambiae* in this region is believed to be due to the use of long term indoor residual spraying (IRS) with insecticide (Reddy *et al*., 2011).Similarly, reduced endophilic preference of *An. gambiae* s.l. post-IRS intervention was reported in Ethiopia by (Lelisa *et al.*, 2017). A zoophilic and exophilic tendencies of *An. arabiensis, An. funestus* s.l was also reported in the country (Gone *et al*., 2014). In Kenya *An. gambiae* s.s. showed a dramatic change from endophilic to exophilic behavior after the implementation of LLIN use. Despite the species' new behaviour, *An. gambiae* s.s. also remains highly anthropophilic, increasing the risk of malaria transmission (Sokhna *et al*., 2013).

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.

Resting behavior of mosquito can be determined also by environmental conditions. For instance, resting behaviour of *An. demeilloni* was shifted from exophilic in the lowland to endophilic in the highland. This could be suggested as temperature affects the biology of anophelines. Thus, they can avoid 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 deposit two basic types of eggs: Rapid-Hatch (RH) and Delayed-Hatch (DH). Rapid-Hatch eggs are deposited directly into water, on the water surface, or on substrate close to the water and usually hatch within 48 h. The RH eggs are laid individually, in small groups, or in rafts containing up to several hundred eggs. Delayed Hatch eggs are usually deposited singly or in small groups, are drought-resistant, survive for long periods out of the water, hatch soon after being re-flooded, and sometimes enter a photoperiod-induced diapause to survive temperate and arctic winters (Day, 2016).

Mosquitoes utilize a wide range of aquatic niches for oviposition, including natural ponds, puddles, stream fringes, marshes, tree-holes and plant axils, man-made pits, drains, rice fields, and containers. Selection of suitable oviposition sites is a critical step in the life history of mosquitoes (Herrera-Varela *et al*., 2014). Oviposition site selection is the net result of the interaction of a complex array of both chemical and physical factors.

Chemical cues may be sensed before physical contact with the site, or they may be sensed upon contact and may emanate 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).

Organisms without any parental care are able to select habitats based on a set of innate or learned cues in order to maximize the survival and fitness of their offspring. Habitat selection is of major importance for the determination of spatial and temporal distributions of populations, and for understanding intra and inter-specific relations that influence the abundance of individuals (Herrera-Varela *et al*., 2014). Once female mosquito took blood meal, it undergoes physiological

changes that shift its host seeking behavior into oviposition site selection. To identify and discriminate among potential oviposition sites, female mosquito use visual, olfactory, gustatory and chemo-tactile cues. Of these olfactory cue which include pheromes and kairomomes is the one mosquito rely to identify suitable oviposition site (Wondwoson, 2016). Vegetation cues associated with larval habitats are instrumental in the oviposition site choice of the *An. arabiensis*. Identifying volatile cues from grasses that modulate gravid malaria mosquito behaviors has distinct potential for the development of tools to be used in future monitoring and control methods. Demonstrated hierarchical preference of gravid *An. arabiensis* for grass volatiles indicates that vegetation cues associated with larval habitats are instrumental in the oviposition site choice of the malaria mosquitoes. Identifying volatile cues from grasses that modulate gravid malaria mosquito behaviours has distinct potential for the development of tools to be used in future monitoring and control methods (Asmare *et al*., 2017).

2.5. Malaria vector control

Vector control can be defined as any kind of measure taken to limit the ability of vector to transmit diseases (Karunamoorthi, 2011). Historically, vector control has resulted in a successful malaria eradication in various parts of the world (Raghavendra *et al*., 2011). Malaria vector control strategies target both the immature and adult stages of Anopheles populations. The interventions include chemical control using IRS and LLINs, biological, genetical, botanical and environmental managements (Sougoufara *et al*., 2017).

2.5.1. Indoor Residual Spray

Indoor residual spray (IRS) is the application of a long-lasting, residual insecticide to potential malaria vector resting surfaces such as internal walls, eaves and ceilings of all houses or structures (including domestic animal shelters) where such vectors might come into contact with the insecticide (WHO, 2015). IRS acts both through repelling mosquitoes from entering houses and by killing female mosquitoes that are resting inside houses after having taken a blood meal. This implies that IRS is most effective against endophilic mosquitoes (Pluess *et al*., 2010). The use of IRS has a decade's history in malaria vector control. The use of dichloro-diphenyl-trichlorethane (DDT) had resulted in the elimination of malaria in Asia, Russia, Europe, and Latin America (Pluess *et al*., 2010). IRS is widely used in areas of seasonal transmission, including epidemic-prone areas, and increasingly in more malaria-endemic areas. It is applied in epidemiological

settings where vectors mainly stay indoors, and in countries where the necessary logistical capabilities can be deployed (Karunamoorthi, 2011).

The percentage of the population at risk protected by IRS declined globally from a peak of 5% in 2010 to 3% in 2017. In Western Zambia IRS has resulted in lower malaria incidence (82/1000 and 400/1000) compared to unsprayed area (398/1000 and 773/1000) both at the beginning and pick of malaria transmission season (Phiri *et al*., 2015). It also declined malaria prevalence in different African countries including Malawi, West Africa, North western Tanzania, Northern Uganda, Western Kenya and Ethiopia (Hamusse *et al*. 2012; Skarbinski *et al*., 2012; Mashauri *et al*., 2013; Phiri *et al*., 2015; Gimnig *et al*., 2016; Tukei *et al*., 2017). The residual efficacy of IRS varies with spray quality and surface wall type. Painted wall surfaces and spraying following the standard guideline resulted in a better residual efficacy (Zerihun *et al*., 2018).

2.5.2. Long-lasting Insecticide Treated Nets

Long-lasting insecticidal mosquito nets (LLINs) are ready-to-use pre-treated mosquito nets, which do not require any re-treatment during their expected lifespan (generally 2–3 years). Data on durability (physical integrity) from the field suggest that they last not more than 24 months (WHO, 2013). LLINs prevent malaria by serving as physical barriers between mosquito vectors and individual users and by killing/repelling the vector as they are impregnated with pyrathroid insecticides (Wanzira, 2016). LLINs are the primary malaria vector control tools in Ethiopia. The major scale up of LLINs was began in the third quarter of 2005 where about 50 million LLINs have been delivered to 10 million homes in malarious areas of the country (FMoH, 2014). According to 2015 national malaria indicator survey report, only 64% of households (HHs) own at least one LLIN, and 40% of the population slept under the LLINs the night before the survey (MIS, 2015). LLINs utilization are found to be influenced by different factors such as low educational level of women, low awareness on malaria prevention, unavailability of separate sleeping room, LLIN color and shape preference (Doda *et al*., 2018; Gobena *et al*., 2012).

2.5.3. Space spraying

Space spraying is the outdoor spraying of insecticides to kill adult insects. The insecticide is dispersed using hand-held, vehicle-mounted or aircraft-mounted equipment to produce a fog. Space spraying is regularly used in public health and pest control programs, including use as an emergency response to malaria epidemics. The appropriate application of space spraying reduces populations of outdoor-biting mosquitoes, and may help reduce malaria transmission from the

mosquito species which are not affected by LLINs and IRS (Pryce *et al.*, 2018). Space spraying is a mainstay of high income countries (HIC) mosquito control programs as a response to disease outbreaks and/or increase in mosquito abundance. It is also experienced by some low and middle income countries including like Turkey, Mauritius and Sri Lanka that have recently achieved malaria elimination. Ground-based space spraying has been employed successfully for malaria vector control in India, Tanzania and El Salvador (Killeen *et al*., 2017). It is effective as a contact poison with no residual effect. Space spray must be timed to coincide with the peak activity of adult mosquitoes, because resting mosquitoes are often found in areas that are out of reach to the applied space spray insecticides (e.g., under leaves, in small crevices).The best time to kill adult mosquitoes by fogging is at dusk, when they are most active in forming the swarms (Raghavendra *et al*., 2011). Generally, space spraying is not cost-effective as a means of malaria vector control, as the operational costs are high and residual effects are low. It may, however, be considered for use in exceptional circumstances, such as emergency situations in refugee camps (Karunamoorthi, 2011).

2.5.4. Mosquito repellent

Insect repellents may be chemical or plant-based substances, preventing from arthropod bites, which eventually help to achieve the reduction of man-vector contact. Repellents are typically applied to exposed skin or can be applied to clothing or other surfaces to discourage arthropods from landing or climbing on to treated surfaces (Karunamoorthi, 2012). Mosquito repellents are currently recommended by the WHO as the first-line malaria-prevention tool for travelers and they are commonly used by expatriates' in tropical developing countries (Maia *et al*., 2018). DEET (N,N-diethyl-3-methylbenzamide) has been the most extensively used personal arthropod repellent for over five decades and it can be applied to exposed skin or clothing (Karunamoorthi, 2012). However, synthetic repellents can cause negative side effects on the environments and human health, reduced efficacy owing to sweating, unpleasant odor, expensive and can cause allergic reactions.

2.5.5. Larval sources management

Mosquito larval source management (LSM) is the management of water bodies that are potential larval habitats to prevent the development of immature mosquitoes into adults (Tusting *et al.*, 2015). It provides a dual benefits in which it can be used to control both indoor and outdoor biting

mosquitoes. LSM is used in large-scale mosquito abatement programmes in North America and Europe (Fillinger & Lindsay, 2011). In Africa it remains as a forgotten and dismissed intervention (Worrall & Fillinger, 2011). However, recently, there is renewed interest in LSM and its practical application as a complementary intervention to LLINs and IRS, especially where outdoor biting by malaria vectors is problematic or where there is resistance to the insecticides used for LLINs or IRS (Olalubi & Chinwe, 2016). Field trials in different eco-epidemiological settings in Africa and Asia have shown that larviciding can reduce the density of adult vectors and consequently malaria transmission and morbidity in settings where mosquito larval habitats are defined (WHO, 2013) but is largely ineffectual where habitats are so extensive that not all of them can be covered on foot and flooding areas (Fillinger & Lindsay, 2011). LSM can be classified as: (1) habitat modification; (2) habitat manipulation; (3) biological control; or (4) chemical larviciding (Tusting *et al*., 2015).

2.5.6. Habitat modification and Habitat manipulation

Habitat modification is a permanent change of land and water. It includes landscaping; drainage of surface water; land reclamation and filling; and coverage of large water storage containers (for example, wells) with mosquito-proof lids and permanent slabs, or complete coverage of water surfaces with a material that is impenetrable to mosquitoes (for example, expanded polystyrene beads). While, habitat modification is a recurrent activity and includes water-level manipulation, flushing of streams, drain clearance, shading, or exposing habitats to the sun depending on the ecology of the vector (Tusting *et al*., 2015).

2.5.7. Biological control

Biological control of mosquitoes is the introduction of natural enemies of mosquitoes into aquatic habitats, for example predatory fish or invertebrates, parasites, or other disease-causing organisms (Tusting *et al*., 2015). Larvivorous fish have been used for over 100 years in mosquito control. *Gambusia affinis* has been widely used to control the immature stages of various vector mosquitoes. Other fish species include *Tilapia* spp. *Poecilia reticulata*, and *Cyprinidae* (Karunamoorthi, 2011). Bacterial agents like *Bacillus thuringiensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) are the most promising larvicidal strains in malaria vector control. *Bacillus* strains are cheap, can be locally manufactured, easily handled, and practically applied. Compared to chemical insecticides, *Bti* and *Bs* showed faster spreading abilities (Kamareddine, 2012). Viruses that infect

mosquitoes can be used either as biological control tools to reduce mosquito populations or to introduce and express foreign genes (transduction) that reduce the vectorial capacity of mosquitoes. Iridescent viruses, nuclear polyhedrosis viruses, cypoviruses, entomopox virus and densoviruses have been reported to infect and kill mosquitoes (Bukhari *et al*., 2013). Use of entomopathogenic fungus belonging to the genera of *Coelomomyces, Culicinomyces, Beauveria*, *Metarhizium*, *Lagenidium*, and *Entomophthora* are considered as promising mosquito control agents. Unlike other infectious agents, fungus does not require host ingestion; external contact with the insect's cuticle is all that is needed to promote an infection (Kamareddine, 2012).

2.5.8. Chemical larviciding

Chemical larviciding is a regular application of synthetic insecticides to larval habitats to control mosquitoes. Currently available insecticides have different modes of action. They include surface films such as mineral oils and alcohol-based surface products that suffocate larvae and pupae; synthetic organic chemicals such as organophosphates (for example, temephos and pirimiphos-methyl) that interfere with the nervous system of larvae; and insect-growth regulators (such as pyriproxyfen, methoprene and diflubenzuron) that interfere with insect metamorphoses and prevent adult emergence from the pupae stage (WHO, 2013).

2.5.9. Botanical based mosquito Control

Although the history of using botanicals has not been mapped very well, evidences from various existing historical sources indicated that the use of some plants in protection against insects date back more than 3000 years in Europe (Pavela, 2016). People experienced various modified parts of some aromatic plants and their extracts particularly as repellents against troublesome insects, ectoparasites and anti-helmintics (Isman, 2006; Benelli *et al*, 2015). Several primate species have been observed anointing their fur/coat by rubbing it with millepedes nad plants like *Citrus* spp., *Piper marginatum*, and *Clematis dioica* (Leal *et al*., 2007).

In Ancient Rome, granaries were often fumigated with various aromatic plants (for example, rosemary, myrrh, and juniper). Aromatic plants were also hung near the entry openings of the granaries. As a result, people learned about the repellent effects of aromatic plant substances (Dubey, 2010). Traditionally plants were also used for the protection of stored harvests or foods against storage pests (Isman, 2006; Grzywacz *et al*., 2014) Preserved documents revealed that finely ground chrysanthemum (*Chrysanthemum cinerariaefolium*) flower was used for control of

ectoparasites such as lice and fleas. Other evidences also indicate that the powder of dried flower of pyrethrum plant was used as delousing in children during 400 B.C. (Pavela, 2016). The Romans also recorded methods of repelling flying insects (gnats) that would have included mosquitoes.

In North America, native cultures relied heavily on plants, and many used plants to repel biting insects (Leal *et al*., 2007).The traditional use of botanicals insect control is widespread among the different cultures and communities of the world. In the rural areas of Chinase used burning of some herbs such as Artemisia (Asteraceae) and *Calmus* to keep away mosquitoes and protect cattle from blood-sucking insects.

Traditional use of botanicals are also common in African countries. In South Africa ethno botanical survey revealed that *Lippia javanica* traditionally used to repel mosquito's away (Mavundza *et al*., 2011) while in East Africa *Ocimum basilicum* L. (Labiatae) is traditionally used by Luo communities to drive away mosquitoes, by laying the branches in the house (Seyoum *et al*., 2002). Small scale farmers in Tanzania used fresh smoke of the leaves of *O. suave* and *O .kilimandscharicum* (Lamiaceae), *Azadirachta indica* (Meliaceae), *Eucalyptus globlus*, (Myrtaceae) and *Lantana cammara* (Verbenaceae) as mosquito deterrents (Kweka *et al*., 2008).

In Ethiopia, burning of dried repellent plants is one of the common phenomena to drive away insects and mosquitoes. It is usually performed by using the traditional charcoal stove (thermal expulsion) in the early evenings (Karunamoorthi and Hailu, 2014). Historically, different communities use different plants in various forms to protect themselves against mosquitoes and other insect bites (Mavundza *et al*., 2011). The traditional knowledge and experiences of the repellent plants has been passed from one generation to another chiefly through word of mouth (Karunamoorthi *et al*., 2009). The chemical contents extracted from plant materials can be useful as larvicides, repellents, oviposition attractants, insect growth hormone regulators and deterrent agents. Plant products have been used in many parts of the world for killing or repelling mosquitoes either as extracts or as whole plant (Kweka *et al*., 2008).

Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides and a wide range of plants with larvicidal and adulticidal properties have been used against insects (Bekele *et al*., 2014). Plants possess a wide range of bioactive phytochemicals that are selective, biodegradable, and have minor or no adverse effects on non-target organisms

and the environment, making them potentially appropriate for use in integrated pest management programs (Kweka *et al*., 2016).

Various studies have focused on the use of natural products, especially plant-derived essential oils, as suitable bioactive agents against the larvae of *An. gambiae* s.s. and other mosquito species (Kweka *et al*., 2016). The result of laboratory experiments conducted to evaluate the larvicidal activity of ethanol extracts from leaves of three plants: castor bean (*Ricinus communis*), vinca (*Vinca rosea*) and lantana (*Lantana camara*) under 5 concentrations ranging from 250- 3000 ppm against the 3rd instar larvae of the main mosquito vector of malaria in Sudan *Anopheles arabiensis* revealed that 100% larval mortality regardless of the different concentrations (Taha *et al*., 2011). Hot water and ethanol extract of *R. communis* have been found to cause 99.44 mortality of *An. gambiae* larvae (Ghebriel and Adugna, 2017). Furthermore, Oluyemi (2017) found a good larvicidal activity of methanol leaf extract of *R. communis* against 2nd and 4th instar larvae of Anopheles mosquitoes. Mavundza *et al*. (2013) found ethanol extract of the bark of *Olax dissitiflora* exhibited the highest larvicidal activity with LC_{50} value of 25.24 µg/ml against *An. arabiensis* mosquitoes in South Africa. Ayalew *et al*. (2017) conducted experimental study on efficacy of *Phytolaca dodecandra* seed powder and its extract larvicide activity against *An. arabiensis* and found 50 mg/l dose of the powder causes 100% mortality with LC_{99} 121.077 mg/l, while 50 mg/l of the crude extract results in 80% mortality with LC_{99} of 616.461. However, the result of Owiti *et al.* (2015) indicated that ethanol extracts of mature green fruits of Endod sourced from Eldoret cause the highest mortality of 56% against *An. arabiensis* 4th instar larvae which is less than the WHO threshold of >80%.

Several plant extracts have been found to be effective in killing of adult mosquitoes. Massebo *et al.* (2013b) studied the adulticidal activities of essential oils of eleven plants namely *Chenopodium ambrosioides, Eucalyptus citriodora, Eucalyptus globules, Lippia adoensis, Mentha spicata, Nigella sativa, Ocimum lamiifolium, Ocimum suave, Piper nigrum, Schinus molle* and *Thymus vulgaris* against a laboratory colony of *Anopheles arabiensis* in Ethiopia. Of these *O. suave* was found to be toxic at low concentration (LC_{50} = of 0.0014 ml% v/v; LC_{90} = 0.0027 ml% v/v). The next efficacious oil was that of *T. vulgaris* with LC_{50} and LC_{90} values of 0.0028 ml% v/v and 0.005 ml% v/v, respectively. The lowest activity was due to *S. molle, E. globulus* and *P. nigrum*.

Soonwera *et al*. (2017) reported the toxic effect of *Z. limonella* oil towards mosquitoes. The highest activity with 100% adult mortality was found in 10% concentration against the adults of *Ae. aegypti* and *C. quinquefasciatus* with LC_{50} 6.0% and 5.7%, respectively. Soonwera (2015) also reported adulticidal properties of essential oil of *Cananga odorata* flowers caused high knockdown rates against three mosquito species at 96% (for *Ae. aegypti*), 98.4 % (for *An. dirus*), and 100 % (for *C. quinquefasciatus*), with effective concentration for 50% egg hactching ($EC_{50)}$ values of 6.2, 4.7, and 5.4 %, respectively. Govindarajan and Sivakumar (2014) tested methanol leaf extracts of *Asparagus racemosus* against adult *An. stephensi, Ae. Aegypti* and *C. quinquefasciatus* and results in a dose-dependent mortality. At higher concentrations, the adult showed restless movement for sometimes with abnormal wagging and then died. Leaf extract of *Oreosyce africana* and fruit extract of *Piper capense* have been found to be effective against adult *An. arabiensis* with LC_{50} and LC_{90} values of 18.74, 39.66 ppm and 24.30, 46.32 ppm, respectively (Bekele *et al*., 2014).

Many plant products are also known for their mosquito repellent activities. Karunamoorthi *et al*. (2010) evaluated the repellent efficacy of methanol-leaf extract of Ethiopian traditionally used insect repellent plant viz., Lomi sar [vernacular name (local native language, Amharic); *Cymbopogon citratus* (DC) Stapf. (Poaceae) against *Anopheles arabiensis* at four different concentrations viz., 1.0, 1.5, 2.0, and 2.5 mg/cm^2. His result indicated that *C. citratus* extract has various degrees of repellency impact against *An. arabiensis* providing the maximum total percentage protection of 78.83% at 2.5 mg/cm^2 and followed 68.06% at 2.0 mg/cm^2 for 12 h. Besides, Essential oil of *Juniperus procera* leaves has displayed various degree of repellency at various concentrations against *An. arabiensis*. The skin repellent test at 1.0, 1.5, 2.5, and 5.0 mg/cm2 of *J. procera* offered 100% protection up to 1.32 h, 2.05h, 3.10 and 5.11h, respectively (Karunamoorthi, 2014).Volatiles collected from the headspace of fresh and dried leaves, the smoke from burning the dried leaves of five plant species, *Corymbia citriodora,Ocimum suave, Ocimum lamiifolium, Olea europaea* and *Ostostegia integrifolia*, traditionally used in Ethiopia as protection against mosquitoes were investigated for their repellent activities against host seeking *An. arabiensis* and *Ae. aegypti* mosquitos.

2.6. Description of Test Plants

2.6.1. *Calpurnia aurea* (Common Calpurnia)

Calpurnia aurea (Aiton) Benth (Digitta in Amharic) belongs to the family Fabaceae. It is a small, multi-stemmed tree, 3–4 m tall, occurring widespread in bush land and grassland in sub-Saharan Africa and India. In southern Ethiopia, it is called cheka by the Borana people and is commonly used in traditional medicine to treat diverse medical conditions and parasitic infestation, both in humans and animals (Zorloni *et al*., 2007). *C. aurea* is used for wound healing, treatment of diarrhoea, leishmaniasis, tapeworm, trachoma, scabies, elephantiasis, swellings, and antibacterial and antioxidant activity has been also reported (Eyasu *et al*., 2013). Besides, the plant is considered to have potential activities against lice, maggot and ticks (Korir *et al*., 2014). *C. aurea* exhibits larvicidal activities against *Ceratitis capitata* (commonly known as med fly) (Birhanu & Asale, 2015). The plant is also used as maize weevils protectants (Hiruy & Getu, 2018).

2.6.2. *Clausena anisata* (Horsewood)

Clausena anisata (Wild) Hook .F. ex Benth (Rutaceae)(syn. *Clausena abyssinica* (Engl.) Engl., *Clausena inequalis* (DC.) Benth.) is a tropical shrub or tree up to 10 meters high growing in and on evergreen forests.

The plant is commonly known as Limich (Amharic) and Ulmaayii (Afan Oromo) in Ethiopia, and 'mbiet ekpene' by the Ibibios of Niger Delta region of Nigeria. The plant is traditionally used as effective remedies for worms infections, respiratory ailments, hypertension, malaria, fever, rheumatism, arthritis and other inflammatory conditions, headaches, pains, toothaches, convulsions and others (Okokon *et al*., 2012). It is also traditionally used for stomach ache and snake bite treatment in Ethiopia (Megersa *et al*., 2013; Tamru & Asalfew, 2016).

Clausena anisata essential oils exihibts larvicidal activities against *An. supictus An. stephensi*, *Ae. albopictus*, *Ae. Aegypti* and *C. quinquefasciatus* (Govindarajan, 2010; Govindaraja & Sivakumar, 2013). *C. anisata* leaf essential oils and extracts have been reported to have an effective larvicidal activities against *An. gambiae* s.s. (Ollengo *et al*., 2016).

2.6.3. *Ricinus communis* (Caster bean)

Ricinus communis Linn. is a tall glaborous and glacous annual sometimes shrubby or allmost small tree 2-4m high plant. It is native to the Ethiopian region of tropical east Africa and has become

naturalized in tropical and warm temperate regions through out the world (Lada & Rupali, 2014). It is a fast growing suckering plant which belongs to family Euphorbiaceae. In Ethiopia, *R. communis* grow as annual in the low lands to small tree perennial in the high lands. India, china, Brazil and USA are the major *R. communis* producers globally. *R. communis* oil is non edible and has been used almost entirely for pharmaceutical and industrial applications (Alemaw *et al*., 2014). Medically, *R. communis* have various uses: as antioxidant, anti-diabetic, anti-asthmatic, anti-fertility, antihistaminic, wound healing, hepatoprotective, lipolytic, anti-inflammatory, antiulcer, molluscicidal and insecticidal activities (Jena & Gupta, 2014).

In Ethiopia, traditionally crushed and juice of *R. communis* seed is applied to exposed skin as mosquito, tick and bedbug repellent (Karunamoorthi & Hailu, 2014). Besides, fresh leaves of *R. communis* is used traditionally to treat rabies in the country (Moa *et al*., 2013).

The preliminary phytochemical study of *R. communis* has revealed the presence of steroids, saponins, alkaloids, flavonoids, and glycosides (Jena & Gupta, 2014). Studies have shownt that *R. communis* seed extract exhibited larvicidal effects against C. *quinquefasciatus, An. stephensi* and *Ae. albopictus* (Mandal, 2010). Its leaf extract was also found to have larvicidal activities against *C. pipiens* mosquito and *An. arabiensis* larvae (Taha *et al*., 2011; Dahchar *et al*., 2016; Ghebriel & Adugna, 2017; Aouinty *et al*., 2018).

2.6.4. *Datura stramonium* (Jimsonweed)

Datura stramonium L. is commonly known as Jimson weed or Datura belongs to family Solanaceae. It is 60-120 cm or more tall, branched and pubescent plant (Sayyed & Shah, 2014). *D. stramonium* originates in the Americas but is now found around the world including the warmer regions of North, Central and South America, Europe, Asia, and Africa. *D. stramonium* has both poisonous and medicinal uses. Traditionally it has an important medicinal value throughout the world. Its leaves and seeds are used in different treatment recipes. The leaves of *D. stramonium* are mixed with mustard oil for treatment of skin disorder. Juice of flower petals is used in ear pain. Seeds are used as purgative, in cough, fever and asthma. Seeds are also used for smoking for its narcotic action (Sayyed & Shah, 2014). It has long been known for its hallucinogenic and euphoric effects as well. The weed was dried and smoked for hallucinations and total relaxation.

In Rwanda a leaf infusion is taken as an antispasmodic and to reduce stomach acidity. In Kenya dried and ground leaves and seeds are eaten mixed with fat to treat ringworm. Headache is relieved by rubbing the scalp with leaves or leaf sap. Hair loss is countered by applying fruit sap or leaf

pulp and these also serve to remedy dandruff. In Ethiopia pieces of young fruit are sucked against tonsillitis and sore throat and applied to abscesses and swollen glands. In Kenya and Lesotho the fruit is heated in hot ash and after cooling juice are squeezed and used as ear drops to treat earache. In Zimbabwe an infusion of fruit ash is drunk to treat stomachache. In Ethiopia the smoke of burning seeds is inhaled to relieve toothache, while in Kenya fresh green fruit is applied for this purpose (Mairura, 2014).

Some medicinal uses of the plant includes its anti-inflammatory property of all part of the plants stimulation of the central nervous system (CNS), respiratory decongestion, treatment of dental and skin infections, anti-obesity, anti-viral activity, antioxidative effects and also in the treatment of toothache and alopecia (Devi *et al*., 2011; Miraj, 2016).

Phytochemical analysis showed that the aqueous and ethanolic extract of the stem-bark of *D. stramonium* contained alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides (Al-snafi, 2017). *D. stramonium* has been found to be exhibit a good larvicidal and repellent activities against *Ae. aegypti*, *C. quinquefasciatus*, *An. stephensi* and *An. gambiae* mosquitoes (Tandon *et al*., 2010; Swathi, 2012; Ghebriel and Adugna, 2017).

2.6.5. *Artemisia annua* (Sweet sagewort)

Artemisia annua L., also known as sweet sagewort, sweet wormwood, sweet annie and annual wormwood is a common type of wormwood that originated in Chaina but naturalized throughout the world. It belongs to the family of Asteraceae and has fern-like leaves, bright yellow flowers and a camphor-like scent. It is a plant included in the Chinese Pharmacopeia that has been used since more than 2000 years to cure a broad array of diseases. Currently, *A. annua* is distributed in North America, Europe, Africa, Asia, America and Australia. It is grown for its aromatic and medicinal leaves, which yields artemisinin has been proven to be potent and effective medicine for the treatment of malaria including cerebral malaria and multi drug-resistance *Plasmodium falciparum* (Damte *et al*., 2011).

The plant *A. annua* was first introduced to Ethiopia in early 2001, by a German catholic church around “chencha’ area of Gamugofa. The plant has been cultivated in north Shoa, Wondogenet and Gamugofa; northern and eastern parts of Gojam, northern and southern parts of Gonder, southern parts of Wollo and in Enderta district of Tigray (Muzemil, 2008). *A. annua* has medicinal

uses such as anti-bacteria, anti-periodic, anti-septic and anti-cancer properties. It is also important for malria parasite treatments (Yimer & Sahu, 2016). *A. annua* leaf extract has shown larvicidal activity against *An. stephensi*, *C. quinquefasciatus* and *Ae. aegypti* mosquitoes (Ghosh *et al*., 2012; Masotti *et al*., 2012; Sharma *et al*., 2016).

3. MATERIALS AND METHODS

3.1. Collection of test plants

The leaves of test plants were collected from different areas; *R. communis* and *D. stramonium* (around Addis Ababa), *C. aurea* (Wondogenet), *C. anisata* (Menagesha) and *A. annua* (Arbamich, Chencha District). The plants were identified and authenticated by the National Herbarium (Department of plant biology), College of Natural and Computational Sciences of Addis Ababa University. Voucher specimens were deposited at the herbarium maintained by College of Natural and Computational Sciences of Addis Ababa University, Ethiopia.

3.2. Preparation of crude leaf extracts

Plant extraction was conducted in Traditional and Modern Medicine Research Directorate laboratory of the Ethiopian Public Health Institute (EPHI). The leaves were washed thoroughly and air dried under shade at room temperature. Then, dried leaves were ground separately to powder using grinding mill. The powdered leaf material were macerated with 80% ethanol and methanol in 1:10 (W/V) using Erlenmeyer flasks and placed on orbital shaker (Gallenkamp 5A-4131. England) at room temperature for 72 hours (Tomass *et al*., 2011). The leaf extract of *C. aurea*, *C. anisata*, *R. communis*, *D. stramonium* and *A. anna* were filtered through cotton and subsequently with Whatman filter paper (12.5 cm size). Filtrates were concentrated using rotary evaporator (Buchi RE 121. Switzerland) to remove solvents from the extract. The crude extracts were then collected in small volume beakers and further concentrated on water bath at 40^0C and then stored in deep freeze until used in mosquito larvicidal tests.

3.3. Mosquito rearing

Susceptible colony of *An. arabiensis* were obtained from Public Health Entomology Research Team (PHERT) insectary at the Ethiopian Public Health Institute (EPHI). Mosquitoes were reared using standard procedures. They were maintained at 25±2 ^{0}C and 80±10% relative humidity and 12:12 light and dark photoperiod. Late third instar larvae were used for all the tests.

3.4. Preparation of test and control solutions

Two hundred milligrams of the dried crude extract of each plant was placed in a standard measuring flask and dissolved in 2mL of ethanol and distilled water (80% ethanol extract) and distilled water only (methanol extract) to prepare 20mL of 1% stock solution. 0.1mL of Tween 80 was used as an emulsifier. From the 1% stock solution, concentrations of 50, 100, 150, 200, 250

and 300 ppm were prepared by adding the appropriate volume of dilution. A mixture of 2mL of ethanol and 0.1mL of Tween 80 were made up to 100 mL in a standard measuring flask by adding distilled water to serve as the negative control solution (WHO, 2005).

3.5. Larvicidal bio-assay with crude leaf extract

In the first phase of bio-assay the larvicidal activities of ethanol and methanol crude leaf extracts of *A. annua, C. aurea, C. anisata, D. stramonium* and *R. communis* were screened against 3rd instar larvae of *An. arabiensis* at 300 ppm concentration. Larvicidal bioassay was conducted for 24 hours in glass beakers of 100 mL test solutions with three replicates following WHO guideline (WHO, 2005). Batches of 25 late third instar larvae of *An. arabiensis* were transferred by means of droppers to 200 mL glass beakers each containing 100 mL of water. Small, unhealthy or damaged larvae were removed and replaced. The depth of the water in the beaker was maintained between 5 cm and 10 cm; as deeper levels may cause undue mortality. The appropriate volume of dilution was added to 100 mL water in the beakers to obtain the desired target dosage. Each test was run three times on different days. The test containers were held at 25±2^{0}C and 80± 10% relative humidity with a photoperiod of 12 h light followed by 12 h dark. Larval mortality was recorded after 24 hours of exposure in each concentration of test solutions. Larvae were confirmed dead when they failed to move after probing them with a needle at their cervical region. Larvae which were incapable of rising to the surface within a reasonable period of time when the test solutions were disturbed would be considered as moribund. Moribund larvae were counted and added to dead larvae.

3.6. Determination of LC_{50} and LC_{90} of crude leaf extract of test plants

Based on the preliminary screening ethanol leaf extract of *D. stramonium*, *R. communis* and methanol extract of *C. aurea* and *D. stramonium* were selected and subjected for dose-response bioassay at the concentrations of 50, 100, 150, 200, 250 and 300 ppm. The average mortality after 24 hour were recorded and used to determine LC_{50} and LC_{90} values.

3.7. Column chromatographic fractionation of the crude leaf extract

Crude methanol leaf extract of *C. aurea* was further subjected to bioassay guided fractionation. Crude leaf extract was fractionated by means of column chromatography using silica gel 60 (0.063-0.2mm mesh size). Column chromatographic elution of crude leaf extract was done with solvent systems of gradually increasing polarity using hexane, ethyl acetate and methanol. The following

ratios of solvent combinations were sequentially used in the elution process Hexane: ethyl acetate 100:0, 80:20, 60:40, 40: 60, and 20: 80; ethyl acetate: methanol 100:0, 80:20, 60:40, 40: 60, 20: 80 and 0:100. A measured volume of each solvent combination was collected gradually with a 10 ml syringe and sprayed uniformly by the sides of the glass into the column each time. This measure prevents solvent droplets from falling directly and disturbing the topmost layer of the column as distortion of this layer would result in non-uniform drain of the fractions. Column chromatographic elluents of crude methanol leaf extract of *C. aurea* were collected in separate flasks and examined by thin layer chromatography (TLC). This was done on silica gel plates (Merck, 60 F254) using Hexane/Ethyl acetate in 7:3 ratio as a mobile phase. Visualization and identification of spots that indicate constituents of each elluent was done using an Ultra Violet lamp at a wave length of 254 nm. Finally, elluents having similar constituents were pooled and concentrated using rotary evaporator and placed in deep freeze until used in mosquito larvicidal tests (Ode *et al*., 2011; Tomass *et al*., 2011).

3.8. Preparation of test and control solutions for chromatographic fractions

For the column chromatographic fraction which is not readily soluble in water, 200 mg fraction was placed in standard measuring flasks and dissolved in 2 mL of ethanol and distilled water to obtain 20 mL of 1% stock solution from which test concentrations of 50, 100, 150, 200, 250 and 300 ppm were prepared by adding the appropriate volume of dilution. 0.1 mL of Tween 80 was used as an emulsifier. A mixture of 2 mL of ethanol and 0.1mL of Tween 80 were diluted to 100 mL distilled water in standard measuring flask to serve as a negative control solution for larvicidal bioassays of column chromatographic fraction (WHO, 2005). Larvicidal bioassay was conducted based on the above procedures (section 3.5).

3.9. Phytochemical screening of methanol and ethanol crude extracts of test plants

Crude methanol and ethanol extracts of all test plants were subjected to preliminary qualitative phytochemical screening to test the presence of major secondary metabolites (alkaloids, flavonoids, terpinoids, tannin, saponin and phenols following procedures described by Madike *et al*., (2017) and Bandiola *et al*., (2018).

1. **Test for alkaloids**

Wagner's test

A few drops of Wagner's reagent (iodine solution in potassium iodide) were added to a few mL of

plant extract. A reddish- Brown precipitate confirms the test as positive.

2. **Test for Flavonoids**

Alkaline reagent test

Extract was combined with a few drop of sodium hydroxide solution. The appearance of intense yellow color which turns to colorless on addition of dilute HCl acide indicated the presence of flavonoids.

3. **Tests for Phenol**

Lead acetate test

50 mg of the extract was dissolved in distilled water. The 3mL of 10% lead acetate solution was added. Appearance of a bulky white precipitate indicated the presence of phenolic compounds.

4. **Tests for saponin**

Foam test

50 mg of the extract was dissolved in distilled water and diluted up to 20ml. the suspension was shaken in a graduated cylinder for 15 minute. Formation of 2cm layer of foam.

5. **Test for tannin**

Ferric chloride test

The extract (50 mg) was dissolved in distilled water and few drops of neutral 5% ferric chloride solution were added. The appearance of dark green color indicated the presence of tannin.

Test for terpiniod

About 0.5 g of plant extract in separate test tube was taken with 2 mL of chloroform; 5 mL of concentrated sulphuric acid was carefully added to form a layer and observed for presence of reddish brown color interface to show positive results for the presence of terpenoid.

3.10. Data analysis

Data from all replications were pooled and mean percent mortalities of the late third instar larvae of *An. arabiensis* that were treated with crude leaf extract of the five plants and column chromatographic fractions were determined by analysis of variance (ANOVA) using SPSS for windows, version 20 after 24 hours of exposure. Post hoc HSD test was also used to separate significant means ($p < 0.05$) in larval mortalities among plant extracts and column chromatographic fractions at different concentrations. For crude as well as column chromatographic fractions of leaf extract of each plants, the LC_{50}, LC_{90} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were

determined using dosage mortality probit regression analyses of SPSS program version 20 to determine their larvicidal efficacies (WHO, 2005).

4. RESULTS AND DISCUSSION

4.1. Larvicidal activity of crude leaf extracts of the test plants

Ethanol and methanol crude leaf extracts of *A. annua, C. aurea, C. anisata, D. stramonium* and *R. communis* were tested against 3rd instar larvae of *An. arabiensis* at 300 ppm concentration. The mean percent mortality of larvae after 24 hours are presented in Table 1. All the test plants were found to have a potential larvicidal activities against 3rd instar *An. arabiensis* larvae at the test concentration. The highest mortality was recorded in methanol leaf extract of *C. aurea*, ethanol leaf extract of *R. communis* and ethanol leaf extract of *D. stramonium* with mortality of 100% 93.33% and 91.55%, respectively. However, methanol leaf extract of *A. annua* followed by ethanol and methanol leaf extract of *C. anisata* recorded the lowest activity with mortality of 68.00%, 71.11% and 72.88%, respectively.

Table1: Larvicidal activity of ethanol and methanol crude leaf extract of test plants against *An. arabiensis* at 300ppm

% Mean mortality ±SE		
Plant species	**Solvents**	
	Ethanol	**Methanol**
Artemisia annua	79.11 ± 1.11^{Aa}	68.00 ± 1.15^{Ba}
Calpurnia aurea	85.33 ± 1.33^{Aac}	100.00 ± 0.00^{Bb}
Clausena anisata	71.11 ± 2.08^{Ab}	72.88 ± 1.11^{Aa}
Datura stramonium	91.55 ± 1.69^{Acd}	90.22 ± 0.96^{Ac}
Ricinus communis	93.33 ± 2.10^{Ad}	86.67 ± 1.33^{Bc}
Negative Control	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}

* Each value (% mean±SE) represents mean value of nine replicates.
* Means followed by the same letters within the same row (Upper case) and within the same column (Lower case) are not significantly different (p>0.05)

There was no statistically significant ($p > 0.05$) difference in the larvicidal potential of ethanol and methanol leaf extract of *C. anisata*, and *D. stramonium*. However, there was significant ($p < 0.05$) difference in the larvicidal efficacy of ethanol and methanol leaf extracts of *A. annua, C. aurea* and *R. communis*. Besides, there was no significant difference ($p > 0.05$) in the larvicidal activities between ethanol leaf extract of *A. annua* and *C. aurea* and between ethanol leaf extract of *D. stramonium* and *R. communis*.

The toxicity difference among test plants and extraction solvent suggested that different plants have different phytochemicals which can be extracted by different solvents. High larval mortality caused by methanol leaf extract of *C. aurea* could be due to the presence of high amount of bioactive secondary metabolites. To our knowledge no study has been conducted on the larvicidal activities of *C. aurea* leaf extract against *An. arabiensis* larvae to compare with, however the plant is known for its larvicidal activities against *Ceratitis capitata* and maize weevils protectant activity in Ethiopia (Birhanu & Asale, 2015; Hiruy & Getu, 2018). Besides, the finding of Korir *et al*. (2014) indicates that the plant has potential activities against lice, maggot and ticks.

Ethanol leaf extract of *R. communis* and *D. stramonium* resulted in 93.33 % and 91.55 % mortality, respectively at 300 ppm against 3rd instar *An. arabiensis* after 24 hour exposure Table 1. This is similar with the finding of Ghebriel and Adugna (2017) where ethanol leaf extract of *R. communis* and *D. stramonium* were found to cause 92.22 % and 70.56 % mortality, respectively against *An. gambiae* larvae at 1000 ppm in Eritrea though the concentrations are different. Similarly, Basheer (2014) reported that leaf ethyl acetate extract of *R. communis* achieved 96 % mortality of *An. arabiensis* larvae in Sudan. The study of Sogan *et al*. (2018) also indicated that methanol leaf and seed extract of *R. communis* caused 100% mortality against *An. culicifacies* and *Ae. aegypti* at 500 ppm.

Methanol leaf extract of *An. annua* followed by ethanol and methanol leaf extract of *C. anisata* recorded mortality of 68.00%, 71.11% and 72.88% at 300 ppm, respectively Table 1. Similarly, study from Ogbonna *et al*. (2010) revealed that ethanol leaf and seed extracts of *A. annua* were found to be lethal to larvae, pupa and adult females of *An. gambiae*. Besides, Alanazi (2018) conducted larvicidal activities of ethanol and aqueous leaf extracts of *A. annua* at 5% concentration against *Ae. aegypti* and found 99% and 100% mortality after 24 hour exposure. The difference in larvicidal activities with the current finding could be due to concentration difference, species and locality of the plants. According to the study of Mavundza *et al*. (2013) ethanol leaf extract of *C. anisata* were found to cause 100% larval mortality against 3rd instar larvae of *An. arabiensis* at 500 ppm and 88% at 250 ppm and the current finding also showed that ethanol leaf extracts of *C. anisata* caused 71.11 % mortality at 300 ppm.

4.2. Determination of LC_{50} and LC_{90} of crude leaf extract of selected plants

Ethanol leaf extract of *D. stramonium*, *R. communis* and methanol extract of *C. aurea* and *D. stramonium* were subjected for dose-response bioassay to determine the lethal concentrations and the results are presented in Table 2. Methanol leaf extract of *C. aurea* caused the highest larvicidal activities against 3rd instar *An. arabiensis* with the lowest LC_{50} of 84.85 ppm and LC_{90} of 192.29 ppm followed by ethanol leaf extract of *R. communis* with LC_{50} of 134.52 ppm and LC_{90} of 304.67 ppm. Ethanol and methanol crude leaf extract of *D. stramonium* relatively showed the highest LC_{50} and LC_{90} (LC_{50} = 156.33 ppm, LC_{90}= 362.50 ppm and LC_{50}=167.68 ppm, LC_{90}= 308.60 ppm), respectively.

Table 2: LC_{50} and LC_{90} of test plant leaf extracts against *An. arabiensis* larvae

Solvent	Plant name	LC_{50}	LCL	UCL	LC_{90}	LCL	UCL	X^2 (df^b = 4)
Ethanol	*Datura stramonium*	156.33	132.72	182.25	362.50	288.88	533.23	3.57
	Ricinus communis	134.52	113.24	156.16	304.67	248.49	422.59	5.30
Methanol	*Calpurnia aurea*	84.85	67.51	100.54	192.29	159.44	253.69	2.46
	Datura stramonium	167.68	147.06	188.68	308.60	262.14	404.21	1.33

*LC_{50}-Lethal concentration that kills 50% of the exposed larvae, LC_{90}-Lethal concentration that kills 90% of the exposed larvae, UCL=Upper confidence limit, LCL=Lower confidence limit, X^2 –chi-square, df- degree of freedom

The highest larvicidal activities of methanol extract of *C. aurea* could be due to the presence of screened alkaloid, flavonoids, terpinoids, tannin, saponin and phenolic secondary metabolites shown in Table 5. The larvicidal activities of aqueous leaf extract of *C. aurea* against *Ceratitis capitata* reported by Birhanu & Asale (2015) can support the current finding despite the difference in the lethal concentration arose which could be the result of difference in solvent type and susceptibility of the test species. In a similar way Taha *et al*. (2011) evaluated ethanol extract of *R. communis* against *An. arabiensis* larvae and found the LC_{50} of 282.70 ppm which is a bit higher than the current finding. This difference could be arisen due to difference in environmental factors. In his review, Yang *et al*. (2018) indicates that plant secondary metabolite accumulation is strongly

dependent on a variety of environmental factors such as light, temperature, soil water, soil fertility and salinity, and for most plants, a change in an individual factor may alter the content of secondary metabolites even if other factors remain constant. Aqueous extracts from leaf of the same plant was also tested against 3rd instar *An. arabiensis* larvae and the LC_{50} value calculated was found to be 445.66 ppm (Elimam *et al*., 2009). The lowest LC_{50} in the current study might be related to the extraction solvent. The study of Pandey &Tripathi (2014) indicates that methanol extracts more bioactive compounds than water. In other study Sogan *et al*. (2018) evaluated the efficacy of leaf *R. communis* methanol extracts against *Ae. aegypti* and *An. culicifacies,* and his result revealed that the LC_{50} against *Ae. aegypti* was 191.54 ppm while that of *An. culicifacies* was 65.629 ppm, respectively. Similarly, the LC_{50} of the current finding against *An. arabiensis* was found to be 134.52 ppm. This indicates that leaf extract of *R. communis* has a potential larvicidal activities against mosquito larvae though the level of activities varies depending on variation in extraction solvent and test species. Swathi *et al*. (2012) found 16.07 ppm LC_{50} of ethanol extracts from *D. stramonium* against *An. stephensi* while in the current study the LC_{50} of ethanol extract of *D. stramonium* against *An. arabiensis* was 156.33 ppm. Similarly, petroleum ether extract of leaf powder *D. stramonium* resulted in the LC_{50} of 409.87 ppm against 3rd instar larvae of *Culex* species after 24 hours exposure (Ullah *et al*., 2018). The difference in larvicidal activity may be occurred due to difference in susceptibility of the test species and solvent type.

4.3. Larvicidal efficacy of column chromatographic fractions of methanol leaf extract of *C. aurea*

Crude methanol leaf extract of *C. aurea* was further subjected to bioassay guided column chromatographic fractionation using solvent systems of increasing polarity. Nine fractions were collected during column chromatographic fractionation of the crude extract. Fraction 7, 8, and 9 were pooled based on their thin layer chromatographic result and designated as F7. The larvicidal activity of each fraction was tested separately against 3rd instar larvae of *An. arabiensis* and mean percentage mortality after 24 hours are presented in Table 3.The result of larvicidal activities of column chromatographic fractions of methanol leaf extracts *C. aurea* revealed that F1-F3 exerted a significant ($p < 0.05$) larval mortality against *An. arabiensis* larvae than the negative control in all concentrations but in F4 significant ($p < 0.05$) mortality than the negative control was recorded at 150, 200, 250 and 300 ppm concentration. On the other hand no larval mortalities were recorded in F5, F6, F7 and negative control in all concentrations after 24 hour exposure Table 3. F1 caused

100% mortality at 250 ppm and 300 ppm concentrations while F2 and F3 achieved 98.66% and 100% mortality at 300 ppm, respectively. However, there was no statistically significant ($p > 0.05$) difference of larval mortality among F1, F2, and F3 at 250 and 300 ppm concentrations. Besides no statistically significant ($p > 0.05$) difference of larval mortality was observed in F1 at 150, 200, 250 and 300 ppm concentrations.

Table 3: Larvicidal efficacy of column chromatographic fractions of methanol leaf extract of *C. aurea* against *An. arabiensis*

Fractions	**% Mortality ±SE**					
	Concentrations (ppm)					
	50	**100**	**150**	**200**	**250**	**300**
F1	34.66 ± 2.66^{Aa}	80.00 ± 2.3^{Ba}	94.1 ± 1.33^{Ca}	98.66 ± 2.30^{Ca}	100.00 ± 0.00^{Ca}	100.00 ± 0.00^{Ca}
F2	21.33 ± 1.33^{Ab}	36.00 ± 2.30^{Bb}	74.66 ± 1.33^{Cb}	82.66 ± 1.33^{Cb}	93.33 ± 2.66^{Da}	98.66 ± 1.33^{Da}
F3	22.66 ± 1.33^{Ab}	54.66 ± 1.33^{Bc}	86.66 ± 3.52^{Cc}	96.00 ± 0.00^{Da}	98.66 ± 1.33^{Da}	100.00 ± 0.00^{Da}
F4	0.00 ± 0.00^{Ac}	2.66 ± 1.33^{Ad}	13.33 ± 1.33^{Bd}	30.66 ± 1.33^{Cc}	46.66 ± 2.66^{Db}	49.33 ± 1.33^{Db}
F5	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
F6	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
F7	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}
Negative Control	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ae}	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Ac}

*F1= Fraction 1, F2= Fraction 2, F3, Fraction 3, F4= Fraction 4, F5= Fraction 5, F6= Fraction 6 and F7= fraction 7
*Means followed by the same letters within the same row (Upper case) and within the same column (Lower case) are not significantly different ($p > 0.05$)

There was no previous study conducted on larvicidal activities of column chromatographic fractions of *C. aurea* leaf extract against *An. arabiensis*. However, the current result is similar with the finding of Tomass *et al*. (2011) on different plant where column chromatographic fractions (F1 and F2) of crude methanol *J. curcas* exerted 100% mortality at 125 ppm against 3rd instar *An. arabiensis* larvae. Arivoli *et al*. (2016) also tested the larvicidal activity of nine fractions of *Sphaeranthus indicus* ethyl acetate whole plant extract against vector mosquitoes of *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* and his result revealed that amongst the fractions tested,

fraction 6th showed 100% mortality against third instar larvae of *Ae. aegypti*, *An. stephensi* and *C. quinquefasciatus* at 100 ppm while other fractions resulted in less than 100% mortality. In the current study also F1-F3 exerted the highest larval mortality than the rest. The variation in toxicity of the different fraction suggested that the active compounds are confined to specific fractions based on their polarity. In a similar way Basheer (2014) also conducted bioassay on the larvicidal activities of column chromatographic fractions of leaf ethyl acetate extract from *R. communis* against *An. arabiensis* and the result indicated fractions 3 caused 100 % mortality after 24 hour exposure at 86 ppm.

4.4. LC_{50} and LC_{90} of column chromatographic fractions of methanol leaf extract of *C. aurea*

The average larval mortality of fractions F1-F4 of *C. aurea* methanol leaf extract after 24 hours exposure were subjected to probit analysis for LC_{50} and LC_{90} determination and the results are presented in Table 4. The result revealed that F1 of *C. aurea* methanol extract showed the lowest LC_{50} of 62.51 ppm and LC_{90} of 122.72 ppm followed by F3 with LC_{50} of 82.33 ppm and LC_{90} of 165.02 ppm. However, F2 and F4 showed the highest LC_{50} of 100.37 ppm and 279.44 ppm, respectively than F1 and F3.

Table 4: LC_{50} and LC_{90} of column chromatographic fractions of *C. aurea* methanol leaf extract against *An. arabiensis* larvae

Fractions	**LC_{50}**	**LCL**	**UCL**	**LC_{90}**	**LCL**	**UCL**	**X^2 (df^b = 4**
F1	62.51	48.24	74.73	122.72	102.33	161.02	0.20
F2	100.37	81.61	117.91	228.58	188.96	305.11	4.47
F3	82.33	67.03	96.40	165.02	139.18	210.82	1.67
F4	279.44	239.98	368.75	578.38	417.97	1264.39	0.71

*F1= Fraction 1, F2= Fraction 2, F3= Fraction 3, F4= Fraction 4

*LC_{50}-Lethal concentration that kills 50% of the exposed larvae, LC_{90}-Lethal concentration that kills 90% of the exposed larvae, UCL=Upper confidence limit, LCL=Lower confidence limit, X2 –chi-square, df- degree of freedom

Similarly, F1 and F3 were found more toxic to *An. arabiensis* larvae with LC_{50} of 62.51 ppm and 82.33 ppm in Table 1, respectively than the crude methanol extract with LC_{50} of 84.85 ppm in Table 2.

Though there were no studies on the dose response bio-assay of column chromatographic fractions of *C. aurea* leaf extract, the finding of Tomass *et al*. (2011) indicates column chromatographic fraction F1, F2 and F3 of *J. curcas* methanol leaf extract have highest larvicidal activities against 3rd instar *An. arabiensis* laravae with LC_{50} values of 28.65, 30.40 and 80. 67 ppm, respectively. In the current study F1 and F3 also caused the highest larvicidal activities against the same species of mosquitoes with lowest lethal concentrations. The current results of fractions F1-F3 are similar with the study of Arivoli *et al*. (2015) in which the column chromatographic isolated fraction "D" of *Murraya koenigii* hexane leaf extracts found to have a potent larvicidal activities against third instar larvae of *Ae. aegypti*, *C. quinquefasciatus* and *An. stephensi* with LC_{50} of 35.06, 27.20 and 42.51 ppm, respectively. *R. communis* leaf ethyl acetate was also fractionated by column chromatography and tested against *An. arabiensis* larvae in Sudan. Fraction 3 was found to cause larvicidal activities with LC_{50} of 125 ppm. The highest LC_{50} observed in crude methanol extract of *C. aurea* than its column chromatographic fraction F1 and F3 suggested that working with this fractions can reduce the lethal concentration to achieve the highest efficacy.

4.5. Phytochemical screening of methanol and ethanol crude extracts of test plants

Preliminary qualitative phytochemical screening was conducted for all methanol and ethanol crude leaf extract to test the presence of alkaloids, flavonoids, terpinoids, tannin, saponin and phenols and the results are presented in Table 5. Methanol crude extract of *C. aurea*, showed the presence of alkaloids, flavonoids, terpinoids, tannin, saponin and phenols. Moreover, the presence of alkaloids was identified in methanol extract of *D. stramonium* and ethanol extracts of *D. stramonium* and *R. communis*. The presence of terpinoids and tannin were observed in all extracts except for ethanol extract of *C. anisata*. The results of the present study also revealed that the presence of saponin and phenol in the ethanol and methanol extracts of *A. annua*, *C. aurea, C. anisata* and *R. communis* Table 5.

The phyto- constituents are important compounds that can be acted as larvicides to different insects. In support of the current study Umer *et al*. (2013) reported the presence of alkaloids, tannin, flavonoids and saponin in 80% methanol leaf extract of *C. aurea*. Root extract of the same

plant was also reported to have cardiac glycosides, tannins, flavonoids, terpenoids, saponins, steroids, alkaloids and phenolic compounds (Dula & Zelalem, 2018). Other studies on seeds extract of *C. aurea* also reported the presence of major secondary metabolites such as tannins, flavonoids, terpenoids, saponins, steroids, alkaloids and phenolic compounds (Nega *et al*., 2015).

Table 5: Phytochemical screening of methanol and ethanol crude extracts of test plants

Plant name	**Solvent**	**Secondary metabolites**					
		Alkaloids	**Flavonoids**	**Terpinoids**	**Tannin**	**Saponin**	**Phenols**
Artemisia annua	Ethanol	-	+	+	+	+	+
	Methanol	-	-	+	+	+	+
Calpurnia aurea	Ethanol	-	+	+	+	-	-
	Methanol	+	+	+	+	+	+
Clausena anisata	Ethanol	-	-	-	-	+	+
	Methanol	-	+	+	+	+	+
Datura stramonium	Ethanol	+	-	+	+	-	+
	Methanol	+	-	+	+	-	-
Ricinus communis	Ethanol	+	-	+	+	-	+
	Methanol	+	-	+	+	-	+

+= Present, - = absent

The presence of alkaloids, terpenoid and tannin in the extracts of *R. communis* are consistent with finding of Aziz *et al*. (2016). Furthermore, aqueous and ethanolic extract of the stem-bark of *D. stramonium* contained alkaloids, saponins, tannins, steroids, flavonoids, phenols and glycosides (Al-snafi, 2017). The absence of flavonoids and saponin leaf extracts of *D. stramonium* in the present study might be related to the plant parts examined.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

The current study revealed that crude ethanol and methanol leaf extracts of *A. annua, C. aurea, C. anisata, D. stramonium* and *R. communis* have a potential larvicidal activities against 3rd instar larvae of *An. arabiensis*. Crude methanol extracts of *C. aurea*, crude ethanol leaf extract of *R. communis* and *D. stramonium* showed better larvicidal activities with more than 90 % larval mortality after 24 h exposure. On the other hand, crude methanol extracts of *A. annua* and crude ethanol extract of *C. anisata* have lowest larvicidal activities with mortality < 73%. Moreover, column chromatographic fractions (F1and F3) of methanol extracts of *C. aurea* caused the highest larvicidal activities than the crude methanol extract of *C. aurea* with lowest lethal concentrations. This implies that the active metabolites of F1 and F3 fractions of methanol extracts of C. *aurea* are promising for further botanical insecticide developments. Besides, preliminary qualitative phytochemical analysis of methanol leaf extract of *C. aurea* revealed the presence of major secondary metabolite such as alkaloids, flavonoids, terpenoids, tannin, saponin and phenols which have potential insecticidal activities.

5.2. Recommendations

Based on the above conclusion the following recommendations have been forwarded:

- ✓ Fraction F1 and F3 of methanol extracts of *C. aurea* can be considered for further mosquito insecticidal developments.
- ✓ Further study need to be conducted to identify the active ingredients in F1 and F3 of methanol extracts of *C. aurea* and their mode of actions.
- ✓ Larvicidal activities and residual efficacy of the extracts should be evaluated under semi-field and operational field condition.
- ✓ Study should be conducted to assess toxicity of test plant extract to non-target organisms.
- ✓ Further study need to be continued up to the formulation of biopesticide from the best performing and locally available test plants.

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