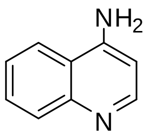
CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Malaria is a life-threatening disease caused by parasitic protozoans *Plasmodium falciparum* and transmitted to human beings through the bite of the *Anopheles* mosquito. Once an infected mosquito bites a person and transmits the parasites, they multiply in the host's liver before infecting and destroying red blood cells (WHO, 2015). If left untreated, malaria can be very fatal thus affected people need treatment to eliminate the parasite from their bloodstream to prevent them from reaching lethal levels (WHO, 2015). This disease is widespread in the tropical and sub-tropical regions such as Sub-Saharan Africa, Asia, and Latin among others (Caraballo, 2014).

Of the many parasitic diseases, malaria is the most dangerous in terms of its wide geographical distribution, high transmission frequency and the extent of morbidity and mortality it causes (WHO, 2015). This is due to the development of resistance by the most lethal causative parasitic species; *P. falciparum* to the mainstay drugs currently in use (*Kaur et al.*, 2009). Thus, the treatment of malaria using 8-aminoquinoline based drugs such as aminoquinoline (**1**), chloroquine (**2**) and amodiaquine (**3**), pyrimethamine (**4**) and mefloquine (**5**) is nolonger effective (WHO, 2015). Substantial efforts have been made towards the development of new active compounds especially from artemisinin (**6**) as an alternative to the mentioned drugs (**1**, **2**, **3**, **4**, and **5**). However, no single drug is available that is effective against multi-drug resistant malaria (Jambuo *et al*., 2005; Wichmann *et al*., 2004). Efforts towards the development of alternative antimalarials from the available ethno medicinal knowledge remain imperative, since there are numerous unexplored botanical remedies.



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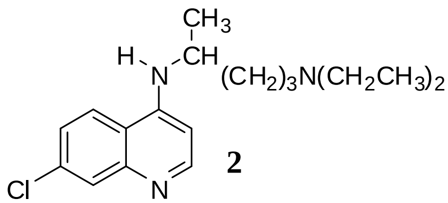

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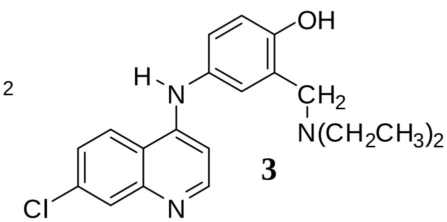

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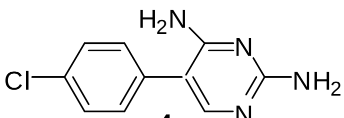

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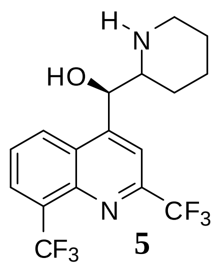

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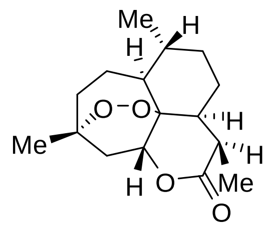

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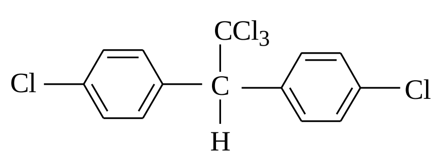
The emergence of multidrug-resistant parasites is a major concern for malaria control and management (WHO, 2015). Also contributing to these challenges is the development of resistant vector mosquitoes (Curtis, 1994). The worst documented example of this scenario is the failed attempt to eradicate the vector with the once successful synthetic insecticide dichlorodiphenyltrichloromethane (DDT) (**7**) (Curtis, 1994). Apart from resistance developed by mosquitoes, DDT is toxic and non-biodegradable, thus its continued use is associated with environmental toxicological effects (WHO, 1996; Curtis, 1994). As such, despite their easy availability and low cost, the use of DDT and related compounds has been restricted to indoor residual spraying (WHO, 2015).



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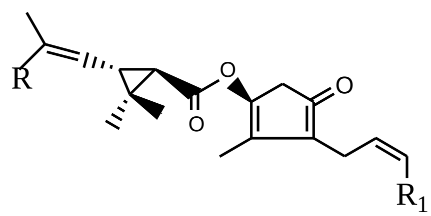
It is therefore imperative that new drugs with unique mechanism of action are urgently needed. Efforts to develop alternative larvicides and mosquitocides have focused on indigenous plants, under the assumption that naturally occurring insecticides may be specific for target organisms and are biodegradable (WHO, 1996). Thus plants have been visualized as "naturally occurring chemical factories," synthesizing metabolites that have potent insecticidal activities (WHO, 1996). This embeds on the notion that so-obtained compounds may have wide range of activities, different modes of action and may not suffer insect resistance. Pyrethrins (**8-12**) isolated from the flowers of *Chrysanthemum cinerariaefolium* (Asteraceae) are plant-based household insecticides and are among in the 174 typical examples of naturally occurring insecticides (Sexana, 1990).



		R	R_1
Pyrethrin I	**8**	CH_3	$CH{=}CH_2$
pyrethrin II	**9**	$COOCH_3$	$CH{=}CH_2$
Cinerin I	**10**	CH_3	CH_3
Cinerin II	**11**	$COOCH_3$	CH_3
Jasmolin	**12**	CH_3	C_2H_5

The use of traditional and herbal remedies as alternative choice in disease management in countries where malaria is endemic is common and Kenya is not an exception. Since several plant species are used in the management of malaria, *Lonchocarpus eriocalyx* (Harms), *Alysicapus ovalifolius* (Schumach), *Erythrina abyssinica* (DC) have been considered in this study with the hope that their antimalarial constituents will be unraveled.

However, scientific validations of ethnopharmacological claims should be based on their biological and phytochemical evidence. In majority of medicinal plants, such evidence is lacking. For instance, the root decoction from *Lonchocarpus eriocalyx* (Harms) is claimed to be used to treat fever, malaria, typhoid and as mosquito repellant by the Embu-Mbeere community (Kareru *et al*., 2007); *Alysicapus ovalifolius* (Schumach) for managing fever by the Miji-kenda, (Kokwaro, 2009); whereas concoctions from *Erythrina abyssinica* (DC) are used to manage malaria by the Luos amongst other communities (Kokwaro, 2009, Kamat *et al*., 1981). However, result of a strategically planned investigation to establish the basis of these ethno-medicinal claims is still scanty and in some plants there is completely no evidence.

Malaria has remained in focus, receiving heightened attention, thus making its control a central element of the Millennium Development Goals (MDGs) (WHO, 2015). It differs in its characteristics from country to country and its geographical distribution (WHO, 2015). This variation is due to climatic conditions such as rainfall, humidity and temperature, hence there is no single strategy for its control that is applicable for all situations. However, the three essential elements of malaria management include: chemoprevention and chemoprophylaxis which suppresses blood-stage infection, vector and larval control which reduces transmission of parasites from humans to mosquitoes and back to humans and case management which involve prompt diagnosis and treatment upon infection (WHO, 2015).

Chemoprevention is mostly applied to expectant mothers and children under 5 years of age (WHO, 2015). For expectant mothers, it involves intermittent treatment whereby sulfadoxine-pyrimetnamine (SP) is administered during antenatal period (WHO, 2015). This is a combination of antimalarial drug containing the sulfonamide antibiotic sulfadoxine (**13**) and the antiprotozoal pyrimethamine (**4**) (WHO, 2015). For children under 5 years of age, amodiaquine (**3**) and SP are administered regularly to ensure that therapeutic concentrations in the blood is maintained (WHO, 2015). Chemoprophylaxis is one method used to prevent infection of the disease, but if an individual is already suffering or if the parasites evade the prophylactic drug, then treatment is absolutely necessary in order to save lives (Kigondu, 2007). Nevertheless, *P. falciparum*, has developed resistance to the commonly prescribed drugs as already been mentioned rendering this management strategy occasionally ineffective (WHO, 2015, 2005).

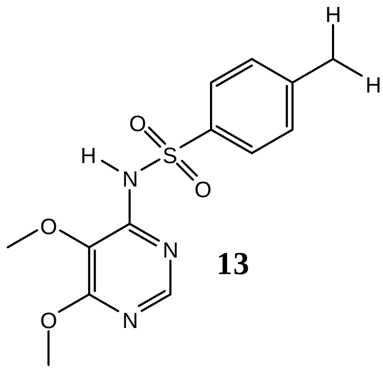
Anopheles gambiae is the most versatile malaria vector in the African Continent and beyond (Bruce-Chwart, 1993). Environmental Management Strategies (EMS) such as covering open water surfaces, filling ditches, clearing of ponds reduces breeding sites which contribute significantly to malaria reduction (Bruce-Chwart, 1993). Use of insecticides is also key to mosquito control and includes spraying of accepted chemical substances with ability to eradicate the vector onto the water surface to form a thin film that prevents them from breathing through the surface of the water (Bruce-Chwart., 1993). There is also use of mosquito repellents as in the case of treated bed nets and mosquito coils (Phillipe and Miller, 2002). In fact, use of Insecticide-Treated Mosquito Nets (ITNs) and Indoor residual spraying (IRS) has been found to reduce malaria mortality rates by an estimated 55% in children aged under 5 years and expectant mothers in sub-Saharan Africa (WHO, 2015). Although the use of commercial insecticides is effective, they are associated with environmental toxicological effects upon prolonged use (Yang *et al*., 2002).

Uses of organolarvicides have been adopted as a control strategy at the larval stages of development of the vector which has proved to be an efficient way of controlling malaria transmissions (WHO, 2015; Bruce-Chwart, 1993). Common habitats for larvae include stagnant waters, ponds, pools and dams which can be targeted and regularly sprayed. The use of synthetic organophosphate larvicides like methoprene, temephos among others has been successful (Gratz and Pal, 1988). However, repeated use of these chemicals has been associated with environmental toxicological effects since they persist, accumulate and can travel long distances in the atmosphere leading to disruption of natural ecosystems (Yang *et al*., 2002). Phytochemicals from plants such as *Lonchocarpus eriocalyx* (rotenoids), *Azadirachta indica* (limonoinds), *Nicotiana tabacam* (alkaloids) and pyrethrum (pyrethrins) have proved to be effective larvicides used to control the populations of the mosquitoes implying that plants are a natural source of these potent chemicals (WHO, 2015, Kareru *et al*., 2007, Bruce-Chwart, 1993).

In search of more effective, affordable and biodegradable alternatives for the control of mosquito vector, plant extracts and pure isolates have been tested for larvicidal activities (Nkya, *et al*., 2014, Yenesew *et al*., 2006). For example, rotenone (**14**) is one of the most extensively used natural larvicide/insecticide and has been reported to be highly active against fourth-instar larvae of *Aedes aegypti* (Kiplagat, 2006). Compound **14** and other rotenoids are known to occur in the genus *Lonchocarpus* hence a potential source of natural larvicides (Caboni *et al.*, 2004). Therefore *L. eriocalyx*, can be considered to have a rich folkloric reputation as a mosquito repellent, however, the potential of the similar compound which may occur in *L. eriocalyx* have not been established. *A. ovalifolius* is used traditionally to treat malaria-related ailments while *E. abyssinica* is known to have antiplsmodial activities. Previous investigations of the stem and root bark of *E. abyssinica* have shown that it is rich in isoflavonoids which exhibited antiplasmodial activity against chloroquine-sensitive (W2), chloroquine-resistant (D6) strains of *P. falciparum* (Yenesew *et al.*, 2008; Yenessew *et al.*, 2009). In spite of the elaborate investigation of the stem and roots bark of *E. abysinnica,* there has been no study on the leaves of these plants which are continuously shed off seasonally. Establishment of the phytochemical ingredients of the leaves will thus provide better reservoir for harvesting without threatening the life of the plants as would be for harvesting the roots and stem barks.

Fungal infections cause morbidity and mortality in severely ill patients, but limited drug classes restrict treatment choices (Perlin, 2007). Fungal infections are opportunistic to persons with compromised immunity, especially in HIV infected individuals (Perlin, 2007). Whereas first-line antifungal drugs like echinocandin B (**15**) represent a valuable treatment option for these infections (Perlin, 2007, (Slavin and Sorell, 2011), they are associated with elevated minimum inhibitory concentrations (MIC) values and occasional treatment failures especially for *Candida albicans* (Andes and Marchillo, 2011, Vandeputte *et al.*, 2012). The use of *L. eriocalyx* and *A. ovalifolius* in the treatment of skin diseases, thrush, ring worms among other fungi related ailments have been reported (Gillet *et al.* 1971; Ichimaru *et al.*, 1996; Udeani *et al.*, 1997) whereas the activity of *E. abysinnica* against *Trichophyton mentagrophytes* is also documented, but this was a zooliphic fungus, thus the activity against this organism may not necessarily be representative of human beings. *C. albicans*, *A. niger* and *A. fumigatus* are some of the notorious fungi which have shown multi-drug resistance to the mainstay anti-fungals (CDC, 2006). However, the bioactivities of the extracts and phytochemicals from these plants have not been tested against these classes of fungi.

Bacterial infections can result in mild to life-threatening illnesses such as bacterial meningitis (Howard *et al*., 1994). Common bacterial infections include tuberculosis (TB), pneumonia, ear infections, diarrhoea, urinary tract infections (UTI), and skin disorders whose treatment has not been very successful using the first line antibiotics such as amoxyllin **(16**), erythromycin (**17**), tetracycline (**18**), cephalexin (**19**), ampicillin (**20**) and cephaloridine (**21**) among others (Hawkey and Jones, 2009).



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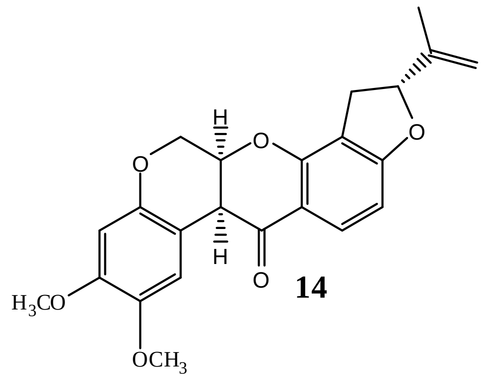

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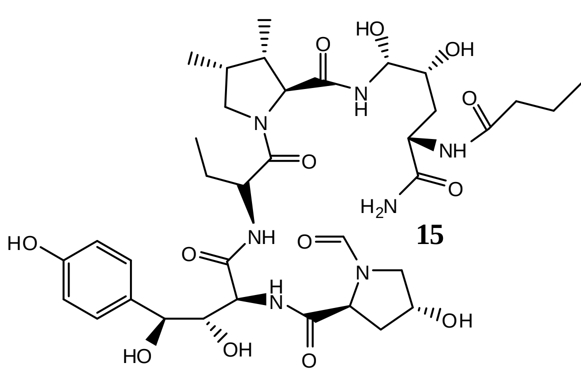
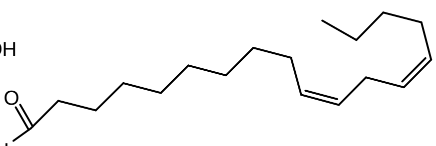

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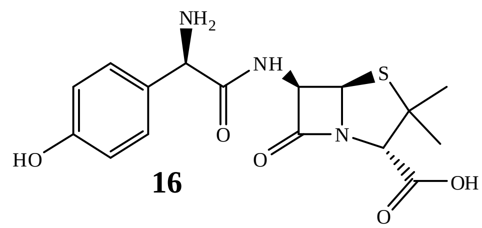

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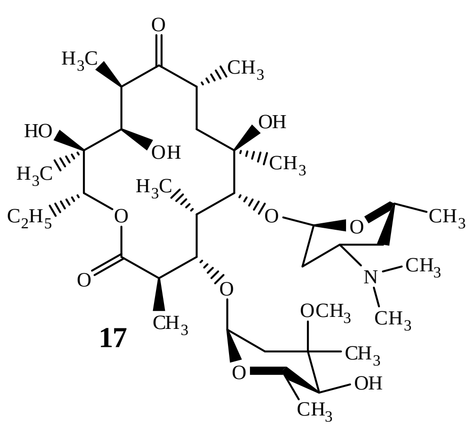

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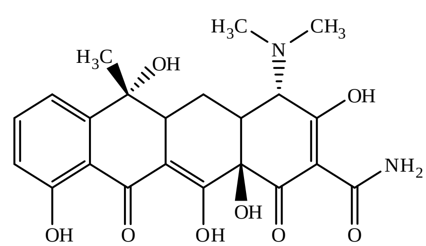

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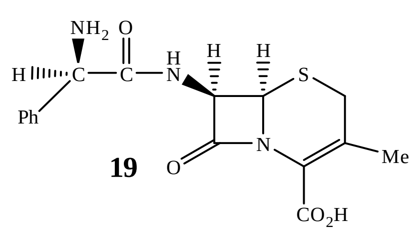

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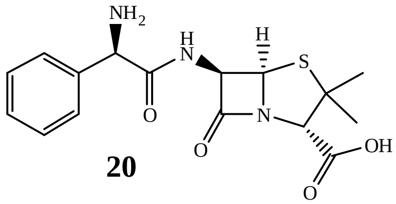

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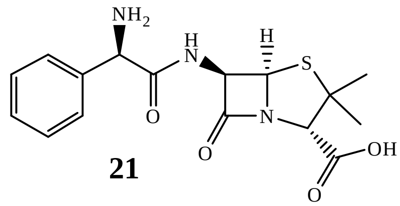

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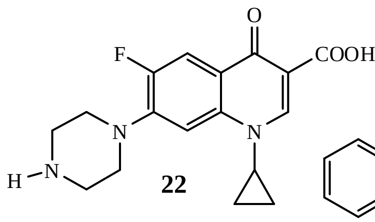
The development of antibiotics in the 1940s offered a powerful tool against bacterial infections that saved lives of millions of people and gave hope for a future in which all bacterial diseases could be controlled (Murray *et al.*, 1998). Decades later, antibiotic resistance is growing relentlessly (WHO, 2010b). For example, fight against new antibiotic-resistant strains of tuberculosis has been reported in Kenya (WHO, 2012c; Sandhu, 2011). Bacteria have an extra-ordinary ability to adapt to environmental challenges such as interactions with antibiotics, both genetically and phenotypically and this contributes to their evolutionary success (Hancock *et al.*, 2011). For example, *Pseudomonas aeruginosa* has high intrinsic resistance to many antibiotics and propensity to develop mutational and/or adaptive resistance to drugs like ciprofloxacin (**22**) and penicillin (**23**) (Hancock *et al.*, 2011). It has consequently joined the ranks of 'superbugs' (Hancock *et al.*, 2011, (Romain *et al*, 2010; Miller and Desai, 2010). The efficacy of first line antibiotics is being lost rendering hospitals as hot-beds for highly resistant pathogens like *Salmonella typhimurium*, *Mycobacterium tuberculosis* and methicillin (**24**)-resistant *Staphylococcus aureus* (MRSA), thus the notion that “hospitalization kills instead of curing” (CDC, 2006).



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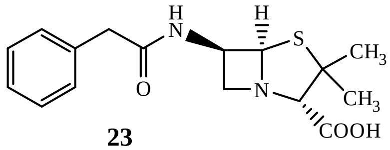

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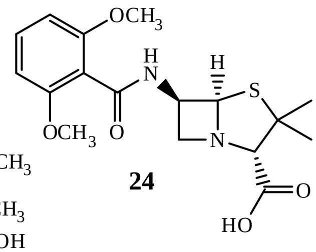

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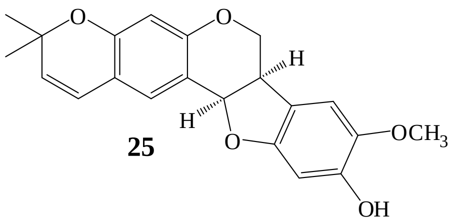
Bacteria therefore pose a significant threat to the general health and welfare of man hence a challenge to researchers. In fact, antibiotic-resistance could bring an “end to modern medicine” since as bacteria evolve to evade antibiotics, common infections could become deadly. In the search for new drugs, medicinal plants remain a potential source for new ideal compounds. Plants from the genus *Lonchocarpus* are used to treat intestinal worms, ulcers, thrush, ring worms (Okello and Ssegawa, 2007). *Alysicarpus* plants are used traditionally as wound medicine and to control cough (Gillett *et al.*, 1971). The phytochemicals responsible for these activities have not been fully identified. Whereas phytochemical constituents of the stem and root barks of *E. abyssinica* are known and their bioactivity, (Irungu, 2012, Yenesew *et al*., 2004), there is no report on the content of leaves. Since this is a very versatile plant as a herbal remedy for addressing various ailments (Kokwaro, 2009, Ichimaru *et al.,* 1996), research on the leaves would unravel the phytochemical constituents and if found to be similar to those in other plant parts, use of leaves would be encouraged to mitigate extinction likely to occur from prolonged use of stem/root and enhance the conservation of this plant.

Phytochemical and biological evaluation of *E. abyssinica* revealed the presence of isoflavonoids such as 8-methoxyneoratenol (**25**) and 5-hydroxy-9-methoxy-10-(3,3-dimethylallyl)pterocarpene (**26**) which exhibited antiplasmodial activity (Yenesew *et al.*, 2008; Yenessew *et al.*, 2009), as well as antimicrobial activities against *S. aureus* and *P.aeruginosa* (Irungu, 2012). However, there is no report of activity tests against other lethal micro-organisms like *Salmonella* *typhimurium*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *E. coli* among others.

Similarly, phytochemical investigations of *Lonchocarpus eriocalyx* yielded, only one compound, lupeol (**27**) which showed antiplasmodial activity (Tuwei, 2006). This implies that extensive and thorough exploration of this plant could unravel more constituent phytochemicals with intriguing bioactivity. Moreover, insecticidal assay was only done against *Aedis egypti*, while its efficacy against the larvae and adult *Anopheles gambie* is not reported (Tuwei, 2006). Some secondary metabolites have been reported from the genus *Alysicarpus* namely; alysinol (**28**) from *Alysicarpus monolifer* (Riaz *et al.*, 2003; Conolly and Hiil, 2005) and threonine (**29**) from *Alysicarpus rugosus* (Siddhuraju *et al*, 1992), stigmasterol (**30**), α-amyrin (**31**) and epicatechin (**32**) from *Alysicarpus bubleurifolius* which showed mild activities against *S. aureus, P. aeuriginosa* and *B. cereus* (Kumar and Nithya, 2014). The ethnomedical use of *Alysicarpus ovalifolius* is reported, but it has not been evaluated phytochemically and biologically.



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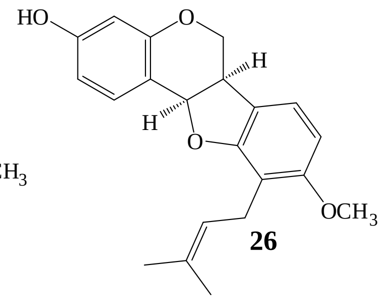

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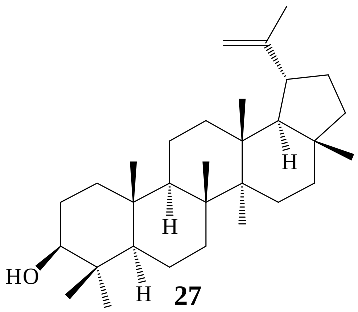

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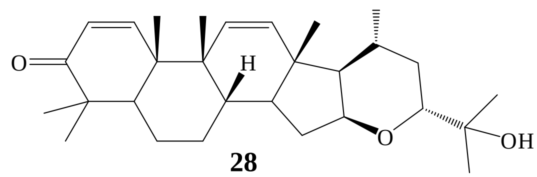

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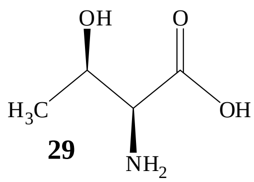

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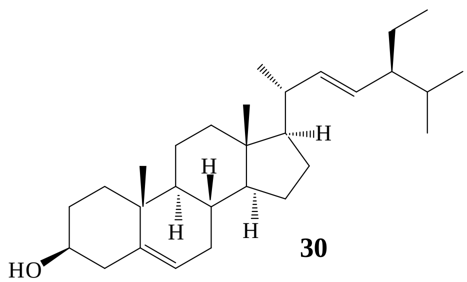

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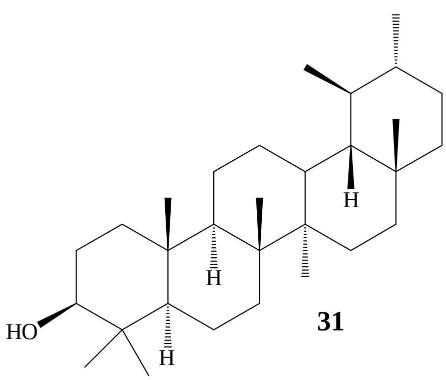

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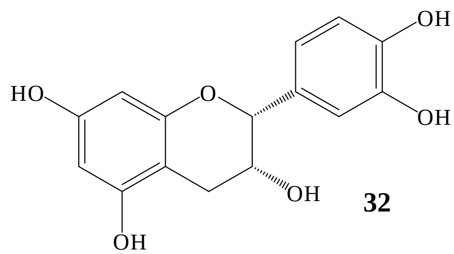

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1.2 Statement of the Problem

Management of diseases continues to be a burden and a major public health problem today mostly due parasitic diseases. Malaria is the most lethal; hence strategies aimed at addressing alternative management tools must take an approach in which the parasite and the vector are all targeted. Fungal infections represent another class of parasitic disease that are opportunistic to persons with compromised immunity, are very aggressive and first spreading, yet the present conventional drugs are associated with occasional treatment failures. Similarly, antibiotic resistance has become a major public health problem within the lifetime of most people such that a number of bacteria are no longer susceptible to drugs that have been used to control them. The use of botanical remedies has existed with human kind for decades as alternative health care systems. For instance, applications of different parts of *Lonchocarpus eriocalyx* (Harms), *Alysicarpus ovalifolius* (Schumach) and *Erythrina abyssinica* (DC.), has been claimed against plasmodial parasite, bacterial and fungal pathogens at the same time mentioned as irritants against mosquitos. Root and stem decoctions of *L. eriocalyx* is used by the Embu-Mbeere people to treat malaria, typhoid, thrush, ringworms, fever while the stem bark is burnt to repel mosquitoes. Root concoctions of *A. ovalifolius* are used by the Miji-kendas to treat fever, thrush and cough, while concoctions from *E. abyssinica* is used by the Luos to manage fever, malaria, syphilis and fungal infections. Despite the traditional use of *L. eriocalyx* and *A. ovalifolius* as herbal remedies, their secondary metabolites, bioactivities and efficacies have not been fully investigated. Although the phytochemistry and biological evaluation of the stem and root barks of *E. abyssinica*, is well documented, there is no report on the leaf extracts.

1.3 Research objectives

1.3.1 General objective

To establish the antiplasmodial, larvicidal, mosquitocidal, and antimicrobial activities and the active compounds from *Lonchorcapus eriocalyx* (Harms), *Alysicarpus ovalifolius* (Schumach) and *Erythrina abyssinica* (DC).

1.3.2 Specific objectives

(i) To extract and carry out bioassays using the crude extracts of *L. eriocalyx* (*stem bark, leaves and roots*), *A. ovalifolius* (*stem bark, leaves and roots*) and *E. abyssinica* (*leaves*) for antiplasmodial, larvicidal, mosquitocidal antimicrobial activities.

(ii) To isolate and characterize compounds from the active extracts of the samples in objective (i) above.

(iii) To determine the antiplasmodial, larvicidal, mosquitocidal, and antimicrobial activities of pure isolates obtained by objective (ii) above.

1.4 Null hypotheses (H_o)

(i) The crude extracts of *L. eriocalyx, A. ovalifolius* and *E. abyssinica* do not exhibit antiplasmodial, larvicidal, mosquitocidal, antifungal and antibacterial activities.

(ii) Pure compounds from the active extracts of *L. eriocalyx, A. ovalifolius* and *E. abyssinica* cannot be isolated and characterized.

(iii) The pure isolates from *L. eriocalyx, A. ovalifolius* and *E. abyssinica* do not have any activities against the test micro-organisms.

1.5 Justification of the Research

The current strategies and interventions for management and control of parasitic diseases are almost out-paced by drug resistance experienced from these organisms. In addition, these drugs are also associated with adverse side effects. This calls for concerted research efforts geared towards generating potent alternatives with hope that these new leads could have different modes of action. The plants under investigations have been claimed and used without proper scientific validation for their efficacies. Scientific evidence would therefore validate the ethno-medicinal claims and enhance their applications.

1.6 Significance of the Research

This study provides vital information that would inform the appropriate use of these plants in health management systems. This will supplement the various interventions aimed at fighting drug resistance hence contribute towards the improvement of health and well-being of humanity. In addition, the study scientifically validates the chemotherapeutic agents hence might unravel the bioactive constituents of *L. eriocalyx*, *A. ovalifolius* and *E. abyssinica*. These compounds could be developed into alternative antiplasmodial, larvicidal, mosquitocidal and antimicrobial agents or used as templates for designing new derivatives with improved properties. If therapeutic efficacy of these plants is scientifically proved, they can be cultivated on large scale to constantly supply the drugs and also for commercial purposes. This will be an income-generating activity for the rural poor and supplement the strategies aimed at poverty alleviation.