A REVIEW ON ANTIPLASMODIAL POTENTIAL AND QUANTIFICATION OF ALOIN AND ALOE-EMODIN IN ALOE VERA

BY

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DECLARATION

I declare that this research project is my original work and has not been submitted elsewhere for examination, a ward of a degree or publication. Where other people's work or my own work has been used; this has properly been acknowledged and referenced in accordance with the Busitema University requirements.

Signature..... Date.....

OTIENO PHELIX OMULLO

APPROVAL

This undergraduate research project report has been submitted for examination with my/our approval as research supervisor(s).

Signature..... Date.....

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DEDICATION

I dedicate this piece of work to my beloved farther, The Late Mr. Omullo Walter Masuwi and mothers, Auma Marita Omullo, Akinyi Jackline Omullo, Atieno Rose Omullo and my wife Auma Queenter Phelix who have done great work to ensure that I reach this far. Their love, care and support cannot be measured may the almighty God bless them abundantly.

I also want to dedicate it to Mr. Bwire Tadeo who supported me in all ways at all times. May the almighty God bless you and finally to all my brothers and sisters and my relatives who supported me spiritually, morally, financially and encouraged me to through this program.

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ABSTRACT

Aloe Vera is widely bused locally in communities in Uganda as a medicinal plant. It is said to contain various nutrient substances and vitamins that have curative properties. It is said to heal a variety of diseases in various communities. However, the extent of use this potential medicinal plant in Uganda and the various ailment for which it is used and the treatment outcome are not clearly established and documented. in this cross-sectional study, carried out in August 2021 in Nagongera sub-county in Tororo District in Eastern Uganda, data was collected from 131 randomly selected adult's respondents using an interviewer administered semi-structured questionnaire. key informant's interviews and focused group discussions were also carried out with purposively selected participants.

Data were collected on social demographic characteristics, practices and beliefs about aloe Vera. the data were analyzed using Excel version 2007 and Epi-Info software. To get the proportion of the community that use aloe Vera, the number of the respondents that use aloe Vera, was expressed as a percentage of the total number of respondents. It was found out that all the respondents (100%) know aloe Vera plant,96.1%think that it can cure and 84.7% have ever used it.

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1.O Background

Throughout the history of mankind, malaria has been one of the major causes of human illness and death. more than 800,000 deaths occur every year, the vast majority being children under the age of five. Thus, this highly infectious disease has a global impact. malaria is parasitic disease widespread in tropical and subtropical regions of the world .it is endemic particularly in regions of Africa, Asia and south America.indias extensive geography and diverse climate supports ideal environment for sustaining malarial parasites and vectors. malaria can be diagnosed easily on morphological basis at different stages of parasite in human blood, with the exception of p. falciparum. P. falciparum is the most severe strain of the malaria due to highest human deaths and resistant to standard anti-malarial drugs (White NH, 2008). the WHO has recommended artemisinin-based combination therapy (ACT) as the first line treatment for multidrug resistant malaria caused by p. falciparum in different parts of the world. Recent studies have reported that p. falciparum has developed resistance to many of the available antimalarial drugs (Richard L.W, 2005).

Malaria has become a leading cause of morbidity and mortality mainly due to its prevalence in poor resource countries, where the therapy is unfordable due to non-availability of oral administered drugs. as antimalarial drug resistance is undermining the effective treatment of the disease, there is a critical need for effective, safe and affordable antimalarial agents (Hamman, J.H, 2008). herbal medicine occupies a pivotal role in treating infectious diseases since onset of mankind. It is estimated that about 40% of all medicines is either natural products or their semi-synthetic derivatives.

Natural products may offer relatively cheap alternative treatment opportunities for malaria patients due to vast metabolic diversity. Currently used antimalarial drugs such as quinine and artemisinin were both isolated from plants cinchona officinalis and Artemisia annua respectively. consequently, it has been established that plants have potential as sources for antimalarial drugs. (Beutler, U, 2007).

Quality evaluation and pharmacological standardization of herbal preparation is a fundamental requirement of industry for commercial production. According to the WHO guidelines, an

herbal product needs to be standardized with respect to safety before releasing it into the market.

Aloe Vera (Aloe Barbadensis Miller) is the most commercialized aloe species belonging to the xanthorrhoeaceae family (Nwaoguikpe, R.N 2010). There are many natural medicinal herbs, but aloe Vera possesses a vast array of healing benefits. Owing to its multipurpose utility, aloe has been introduced into cultivation as a household plant. It has been in use since ages as folk medicine. Aloe Vera is a rich source of over 200 naturally occurring nutrients which contain water soluble and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids. Its secondary metabolites have multiple properties such as anti-inflammatory, antibacterial, antioxidant, immune boosting, anticancer, antiaging, sunburn relief and antidiabetic potentials. Several traditional uses have also been reported such as burn injury, eczema, cosmetics, inflammation and fever (EZZET F, V.V, 2000). aloe juice mixed with water and honey is used as an effective antimalarial cure in Yemen.

Recently Aloe Vera was reported to be used against malaria parasite with the highest frequency in a documentation report on medicinal plants used by local communities of western Uganda. Van Zsyl and Viljoen screened the main constituents of 34 aloe species for antiplasmodial activity using the titrated hypoxanthine incorporation assay.

Aloe Vera is commonly grown and used in most part of Uganda and East Africa. the plant is widely used as a medicinal plant by a number of communities in Uganda.

It is used in various ways, as full therapy or adjunct therapy. Aloe Vera products are used as over-the-counter OTC) medications, or self-medication or self-care, as home remedies, or as dietary supplements, health food, functional foods and many others (Gilbert Onyango February 2007). Despite this long-term native use of this medicinal or potentially toxic and, the use in terms of number of people that use it is not clearly documented. The proportion of the population that benefits from this plant or that is exposed to its toxic effects is not known. (Ifeoluwa T. Oyeyemi, A.A, 2017), this study therefore seeks to determine the extent of use of Aloe Vera plant locally for management of ailments in communities in Tororo District Eastern Uganda.

Tororo is one of the most districts being affected by high malaria death cases due to poor medical conditions and also having many mosquito breeding grounds such as swamps. Therefore, due to increasing population, there is need for these medicinal plants to help cure malaria to the population that are not able to access the modern advanced medication.

To investigate the presence of Alion and Aloe- Emodin in Aloe Vera plant for Anti-plasmodial potential

This research study review was purposefully to provide the knowledge about the presence of Alion and Aloe-Emodin in Aloe Vera. Alion and Aloe-Emodin have proven to be rich sources of novel natural compounds with a wide-spectrum of biological activities and a high level of structural diversity.

2.0 Anti-malarial

Antimalarial are a type of ant parasitic chemical agent, often naturallyderived, that can treat or prevent malaria. The commonest are Quinine, Chloroquine, Hydroxychloroquine, Amodiaquine Pyrimethamine, Proguanil, Sulfonamides, Mefloquinem, Atovaquone, Primaquine, Artemisinin and derivatives, Halofantrine, Lumefantrine, Doxycycline, Clindamycin.

Artemether-Lumefantrine, an artemisinin-derived, remains the best combination anti-malarial accepted by the Food and Drug Administration by 2009 in treating P (Z G, Premji 2009). falciparum malaria. Artemether-Lumefantrine is a combination in the one tabulates Coartem. By 2009, the Food and Drug Administration had permitted artemether-Lumefantrine to treat uncomplicated P. falciparum malaria. The World Health Organization (WHO) recommends artemether-Lumefantrine as a first-line treatment option for uncomplicated P. falciparum malaria worldwide. In contrast, the CDC (Disease Control and Prevention (CDC) malaria hotline) recommends it as therapy for P. falciparum in areas of chloroquine resistance (Bahekar, S, 2013). This combination has tried to limit the problem of drug resistance by the parasites.

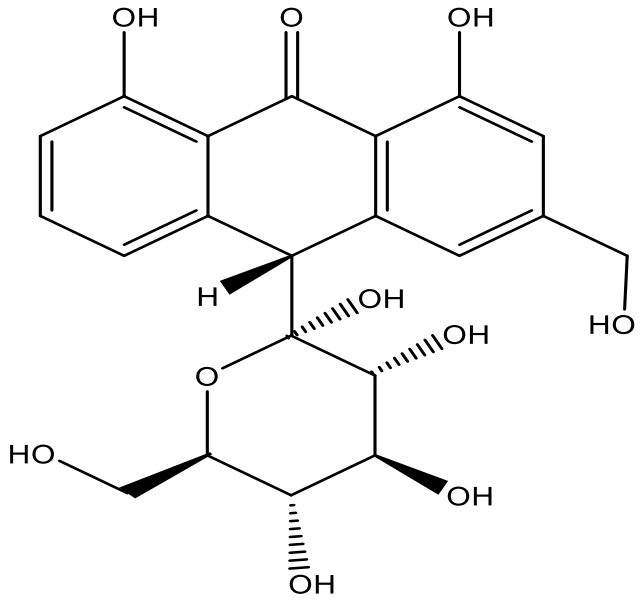
Anti-malarial drug resistance is the capability of a parasite strain to live and reproduce regardless of administration and absorption of a drug given in doses equal to or higher than those regularly recommended but within the limits of tolerance (Yeap, s.k, 2010).

2.1 Aloin

Aloin also known as barbaloin, is a bitter, yellow-brown colored compound noted in the exudate of at least 68 Aloe species at levels from 0.1 to 6.6% of leaf dry weight (making between 3% and 35% of the total exudate) and in another 17 species at indeterminate levels (Luke Mizzil, c.c, 2020). It is used as a stimulant-laxative, treating also constipation by inducing bowel movements. the compound is present in what is commonly referred to as aloe latex that exudes from cells adjacent to the vascular bundles, found under the rind of the leaf and in between it and the gel. When dried, it has been used as a bittering agent in commerce

(alcoholic beverages). Scientific names given include Aloe perryi, A. Barbadensis (=A. Vera), A. ferox, and hybrids of A. ferox with A. Africana and A. spicata. Aloe is listed in federal regulations as a natural substance that may be “safely used in food” when used “in the minimum quantity required to produce their intended physical or technical effect and in accordance with all the principles of good manufacturing practice (Lawrence R, T.P, 2009). “this food application is generally limited to use in quite small quantities as a flavoring in alcoholic beverages and usually be identified only as a “natural flavor”

Figure 1 shows the structure of Aloin



Therefore, Aloin extracted from natural sources is a mixture of two diastereomers, termed Aloin A (also called Barba loin) and Aloin B (or isobarbaloin), which have similar chemical properties (Adama, K.K, 2011). Aloin is an anthraquinone glycosyl, meaning that its anthraquinone skeleton has been modified by the addition of sugar molecule. Anthraquinone are a common family of naturally occurring yellow, orange and red pigments of which many have cathartic properties, attributes shared by Aloin. Aloin is related to Aloe-Emodin, which lacks a sugar group but shares aloin’s biological properties (Ibrahim Kahramano -glu, 2019).

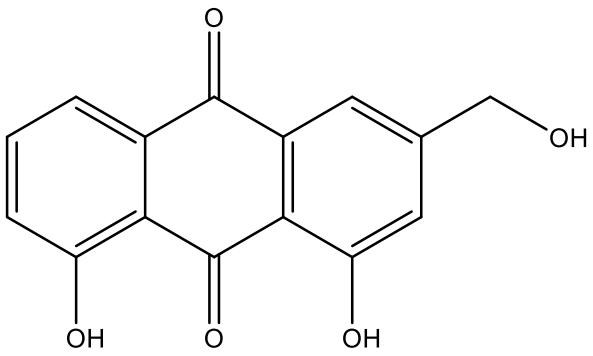
Aloin is usually prepared by extraction from aloe latex, the bitter yellow species of the genus Aloe, especially A. forex, the bitter yellow exudate that seeps out from just underneath the skin of aloe leaves. The latex is then dried and powdered to make the final product, often made into

tablets or a beverage, though Aloin does not have good stability in aqueous solutions. Products derived from the aloe plant do not contain appreciable amounts of Aloin, and have been proven effective for any disease or condition when taken orally.

2.2 Aloe Emodin

Aloe emodin is a naturally anthraquinone derivative and an active ingredient of Chinese herbs, such as Cassia occidentalis, Rheum palmatum L. Aloe Vera and Polygonum multiflorum Thumb. Emerging evidence suggest that aloe-emodin exhibits many pharmacological effects, including anticancer, antivirus, anti-inflammatory, anti-plasmodial and hepatoprotective activities (Yuan Zhang I D, 2018). These pharmacological properties layby the foundation for the treatment of various disease including influenza virus, inflammation, sepsis, malaria, glaucoma, liver fibrosis and several types of cancers. However, an increasing number of published studies have reported adverse effects of aloe-emodin. The toxicity among these reports is hepatotoxicity and nephrotoxicity, which are of wide concern worldwide. Pharmacokinetic studies have demonstrated that aloe-emodin has a poor intestinal absorption, short elimination half-life, and low bioavailability (Z G, Premji 2009). This review aims to provide comprehensive summary of pharmacology, toxicity, and pharmacokinetic of aloe-emodin reported to death with an emphasis on its biological properties and mechanism of action.

Figure 2 shows the structure of Aloe-Emodin



2.3 Aloe Vera

Aloe Vera is a herb distributed all over Uganda and the whole world. Historically aloe implies a shining bitter substance in the Arabic word, where the name Aloe originates from. Botanically Aloe Vera is Aloe Barbadensis Miller, belonging to the Liliaceae family, with about 360 species. It is a cactus-like plant that readily grows in a dry and hot climate and, because of high demand currently, it is cultivated in large amounts. Mainly, it grows in arid regions of Africa. Some medicinal products and Cosmetics are commonly made from mucilaginous tissue at the center of the Aloe Vera leaf called Aloe Vera gel (Dagne. E, 2000). This gel is an explicit, tasteless, thin, jelly-like material.

The remaining part of the plant is known as the pericyclic, which is a group of specialized cells tubules. These occur just under the outer green coat of the leaf. The cells produce bitter yellow liquids that consist of latex with decisive laxative-like action (Richard L.W, 2005). The leaves are arranged in a rosette configuration, triangular with spear-like and have thorny ridges. The plant has yellow flowers.

Approximately 110 potentially active constituents are contained in Aloe Vera from six different classes: chromone and its glycoside derivatives; anthraquinone and its glycoside derivatives; phenylephrine and phenol derivatives; flavonoids; phenylpropanoids and coumarins; and phytosterols and others. Apart from their medicinal uses, Aloe gels have an essential role in food preservation, primarily as edible coatings (Bobbarala, V 2012). Also, provide an edible barrier primarily for atmospheric gases and moisture. It also helps to reduce the transpiration and respiration of fresh produce. This helps to preserve its postharvest quality.

The health profits associated with Aloe Vera are credited to polysaccharides contained in the gel extracted from the leaves. These biological actions involve antifungal activity, hypoglycemic or ant diabetic effects anti-inflammatory, promotion of wound healing anticancer, immunomodulatory and gastro protective properties, and the essential pharmaceutical applications that include using the desiccated A (Erena B.K, 2017). Vera gel fine particles. Aloe Vera comprises 75 active constituents that can function: sugar, lignin, saponins, vitamins, salicylic acid enzymes, minerals, and amino acids.

2.4 Medicinal uses of Aloe Vera

Aloes have been used as medicinal plants for centuries. Leaves of aloes, especially *A. Vera*, are used in the production of many cosmetic products. This is part of the 'back to nature' movement, whose adherents believe that using natural products derived from plants such as the well-known 'health plant' *Aloe Vera* is a healthy way of life. The many kinds of product on the market include after-shaving gel, a mouthwash, hair tonic and shampoo, skin-moistening gel, and even a 'health drink. The species Aloe L (EZZET F, V.V, 2000). Have been used as ethnic medicines in many different countries for centuries, possessing functions, such as anti-cancer, anti-inflammatory, anti-virus, evacuating, protecting liver, and increasing immunity (Cowan,M. 1999). Different species of Aloes in Tororo are used to treat abdominal problems, fever, chicken and other animal diseases with undisputed success stories.

3.0 Methods for Extraction

The samples were first washed with tap water and then surface sterilized in 10% sodium hypochlorite to prevent the contamination of any microbes. They were thoroughly rinsed with sterile distilled water. The plant samples were shade dried followed by oven drying (50^0C) and milled in an electrical blender (EZZET F, V.V, 2000). Aqueous extract of different accessions were prepared by cold percolation method. The extracts were pooled, and the solvent was evaporated using a rotary evaporator under reduced pressure at 40^0C. The crude extracts thus obtained were kept at 4^0C for antiplasmodial assay.

3.1 Methods for analysis of phytochemicals

HPTLC system equipped with a sample applicator linomat V with CAMAG sample syringe, twin lough plate development chamber (20*10cm), TLC scanner 3 and integration software WINCATS 1.4.8 was used for analysis of aloin and aloe-emodin amount. The antiplasmodial activity of plant extracts was assessed against a chloroquine (CQ) sensitive strain of P. falciparum (MRC-2). Minimum inhibitory concentration (MIC) of aqueous extracts of selected samples was determined according to the World Health Organization (WHO) recommended method that was based on assessing the inhibition of schizont maturation in a 96-well microliter plate. EC (effective concentration) values of different samples were observed to predict antiplasmodial potential of the plant in terms of their climatic zones.

HPLC analysis of ant-malaria agent, chloroquine (CQ) in blood and tissues with a simple HCl back extraction method was applied to three forensic autopsy cases. CQ concetraions in femoralvein blood were 8.5, 48.4 and 48.8 microg/ml in three cases, respectively, which were high enough to attribute the cause of deaths to an acute CQ poisoning. There were great site dependent variations in blood CQ levels (Lawrence R, T.P, 2009). The right heart blood samples were very high, which may be explained by incomplete distribution of the drug before death or postmortem diffusion from liver and its surrounding blood, as high CQ levels were remarkable in the liver.

A sensitive LC-MS/MS assay was developed for the quantitive determination of TK900D. Multiple reaction monitoring (MRM) in the positive ionization mode was used for detection. The analyte and the internal standard (TK900E) were isolated from blood samples by liquid-liquid extraction with ethyl acetate.

Chromatographic seoeration was achieved with a Phenomenex Kinetex C18(100×2.0 mm id, 2.6 um) analytical column, using a mixture of 0.1% formic acid and acetonitrile(50:50;v/v) as the mobile phase . The method was fully validated over concetraions that ranged from 3.910 to1000 ng/ml, and used to evaluate PK properties of the lead compounds in a mouse model.

3.2 Antiplasmodial activity of Aloe Vera

The antimalarial activity of Aloe Vera and isolates was evaluated by suppressive test on plasmodium berghei infected mice. The mice were housed in standard transparent cages and maintained on pellet and water ad albitum for 7 days to acclimatize the laboratory environment. 25 male mice of 6-8-week age and weighing 20-25g were infected with 0.2 ml blood suspension (about 1×10^7 parasitized RBC) intraperimentoneally and randomly divided into five groups of five mice per group with three experimental groups and two control groups (one for chloroquine as a positive control and the other distilled water or vehicle as a negative control) for each test sample. (Lawrence R, T.P, 2009).

The Aloe Vera was prepared at three different doses at three different doses of 100,200, and 400 mg/kg of body weight of mice and chloroquine at 25 mg/kg in a volume of 1 ml/100 g body weight of the mice. The leaf Aloe Vera or the standard was administered as a single dose per day and given through oral route using standard oral gavage. Treatment was started 3 h after infection on da 0 and was then continued daily for four days in order of their infection time. On the fifth day (D_4), (Z G, Premji 2009), thin smears of blood films were obtained from the tail of each mouse and smeared on a microscope from the first infected mice and then in order of their infection time (Lawrence R, T.P, 2009). Then, the smears were fixed with absolute methanol and stained with 10% Giesmsa solution for 25min. The chemosuppression effect of each TLC isolates was tested using the above-mentioned method which was applied to test the antimalarial activity of Aloe Vera.

Parasitemia level was determined by counting the number of parasitized erythrocytes out of three random fields of the microscope from each slide. Average percent parasitemia and suppression were calculated using the following formula.

%parasitemia = Type equation here.

% suppression= Type equation here.

Body weight and rectal temperature of each mouse in all groups were measured before infection (day 0) and day 4 using electronic balance and digital thermometer, respectively, to observe the effect of the test sample in body weight and temperature of each mouse in all group.

Mortality was monitored daily and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period of 28 days (D_0-D_{27}) for all test samples (White NH, 2008). The mean survival time (MST) for each group was then calculated using the following formula:

MST= Type equation here.

Therefore, the enhanced suppression of parasitemia growth was observed with increasing the dose of the aloin substance in Aloe Vera plant (M. Giday, 2009). Thus, after the administration of the Aloe Vera for 4 days at doses of 400 mg/kg/day, they caused 56.4%, 67.4%, 64.2% and 79.6% suppression of parasite growth respectively, with the corresponding vehicle treated mice which g0t 47.0+or-3.49% percentage parasitemia. The parasite growth inhibitions observed after treatment with latex, AM_1, AM_2, or AM_3 at three different doses were statistically significant ($p<0.001$) when compared to the vehicle treated group (negative control). Chloroquine was observed to absolve the P. berghei infected mice from the parasite on the fifth day of the infection time which was significant ($p<0.001$) when compared to the negative control group. There was also statically significant difference in the growth inhibition activity of chloroquine as compared with aloin in Aloe Vera leaf and TLC isolates excluding AM_3 at the highest dose.

Mean survival time (MST) of P. berghei infected mice was also recorded to evaluate an antimalarial activity of test substance. The mice treated with the Aloin and isolates of A. meglacantha at three different doses were observed to survive for longer time than the negative control group in a dose depe-dependant manner.

Another parameter that helps to identify antimalarial activity of the leaves Aloin is prevention of body weight loss in day 4 from day 0. Although infection of mice with P. berghei caused about 4.6% body weight loss (as observed in the negative control), treating the mice with both Aloin and TLC isolates was shielded from curtail of their body weight. All doses of both leaves aloin and

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