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BIOLOGICAL ACTIVITIES OF *HILLERIA LATIFOLIA* (LAM.) H. WALT AND *LAPORTEA OVALIFOLIA* (SCHUMACH.) CHEW

By

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DECLARATION

The experimental work described in this thesis was carried out at the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Science, KNUST, Kumasi, Ghana. I hereby declare that to the best of my knowledge this work has not been submitted for any other degree.

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ABSTRACT

Plants have been accepted as part of human culture for the treatment of diseases and ailments, with about 80% of the populace in developing countries depending on plants for medicinal purposes. However, few plants have been scientifically proven for their folkloric uses and assessed for their quality, efficacy and toxicity. The study therefore sought to evaluate the antimicrobial, antioxidant, anti-inflammatory and wound healing activities as well as cytotoxic effects of *Hilleria latifolia* (Lam.) H. Walt. (Family Phytolaccaceae) and *Laportea ovalifolia* (Schumach.) Chew. (Family Urticaceae). These plants are used locally for the treatment of wounds, general oedema and skin diseases. The broth dilution method was used to assess the microbial susceptibility and MIC against typed and clinical strains of Gram-positive, Gram-negative bacteria and fungi. The effect of sub-inhibitory concentration on some antibiotics was also determined. The antioxidant activity was investigated employing the DPPH free radical scavenging assay, the total phenolic content as well as the total antioxidant capacity assays. The carrageenan-induced oedema model in rat was used to evaluate the anti-inflammatory activity of the plants. The wound healing activity was established using the excision wound healing model in rats. The MTT-assay for cell viability and lactate dehydrogenase (LDH) assay were used to assess the cytotoxic effect of the plant extracts. The MICs of *Hilleria latifolia* leaf methanol extract (HLML), *Hilleria latifolia* root methanol extracts (HLMR) and *Laportea ovalifolia* leaf methanol extract (LOML) ranged from 50 to100 mg/mL against test microorganisms. The sub-inhibitory concentration (5 mg/mL) modified the activity of amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin against the test microorganisms by either potentiating or reducing their antimicrobial activity. HLML,

HLMR and LOML exhibited radical scavenging activity with IC_{50} values of 102.5 ± 1.5, 233.5 ± 0.5 and 130.8 ± 0.9 µg/mL. Total phenol content and total antioxidant capacity increased as concentration increased. The total phenol content of HLML, HLMR and LOML was calculated to be 103.0 ± 1.335, 91.32 ± 4.258 and 56.75 ± 0.3220 mg/g, respectively. The total antioxidant capacity was 410.4 ± 4.732 mg/g for HLML, 408.0 ± 18.70 mg/g for HLMR and 337.6 ± 6.961 mg/g for LOML. The antiinflammation activity of HLML, HLMR and LOML at 300 mg/kg caused a significant (p<0.001) decrease in oedema when administered before and after inducing oedema. There was also significant (p<0.001) increase in wound contraction from days 5 to15 after injury when treated with 5 and 10% HLML, HLMR and LOML, with increased fibroblast proliferation and collagenation as well as reepithelialisation. Treatment with 100 µg/mL of HLML (p<0.001) and HLMR (p<0.05) significantly caused a reduction in cell viability, whiles cells treated with LOML showed no significant (p>0.05) difference in viability when compared to the untreated cells. HaCaT-keratinocytes treated with HLML, HLMR and LOML (0.1, 1.0, 10.0 and 100.0 µg/mL) exhibited no significant (p>0.05) LDH release when compared with untreated cells. The study therefore indicated that HLML, HLMR and LOML possess antimicrobial, antioxidant, anti-inflammatory and wound healing activities and they did not exhibit cytotoxic activities at the concentrations used.

LIST OF PUBLICATIONS

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DEDICATION

This work is dedicated to my parents, Very Rev. and Mrs. Oteng Dapaah. Indeed this is the fruit of their labour.

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ABBREVIATIONS

AGEs	Advanced glycation end products
ATCC	American type culture collection
AUC	Area under the curve
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BrdU	5-bromo-2-deoxyuridine
CAT	Catalase
CFU	Colony forming unit
CoQ	Coenzyme Q
DMARDs	Disease modifying anti-rheumatic drugs
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
ECM	Extracellular matrix
EGF	Epidermal growth factor
FCS	Foetal calf serum
FGF	Fibroblast growth factor
FTC	Ferric thiocyanate
GPx	Glutathione peroxidase
HaCaT	Human adult high calcium low temperature
HCL	Hydrochloric acid
HPLC	High performance liquid chromatography
IL-1	interleukin-1
IL-1β	Interleukin-1β
IL-6	Interleukin-6
LDH	Lactate dehydrogenase
LDLs	Low-density lipoproteins
MBC	Minimum bactericidal concentration
MDA	Malondialdehyde
MDRIs	Multi-drug resistance inhibitors
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NSAIDs	Non-steroidal anti-inflammatory drugs
PBS	Phosphate buffer solution
PDGF	Platelet derived growth factor
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TNF-α	Tumor necrosis factor-α
TPTZ	2,4,6-tri(2-pyridyl)-s-triazine

CHAPTER 1

1.0 Introduction

Plants serve as sources of medicine and the use of plants to treat and prevent infectious diseases and other ailments is well accepted as part of culture and traditions of Africans and other parts of the world. This practice has been in existence since time immemorial and up to 80% of the population in developing countries depend on plants for medicinal purposes (Ayyanar and Ignacimuthu, 2011; Calixto, 2005) due to their availability, easy accessibility, low cost and minimum side effects (Akharaiyi, 2011; Tabuti *et al.*, 2003).

About 30% of medicines employed for therapeutic purposes have their source from natural products such as plants (Ramos *et al.*, 2008), making herbal medicine an integral part of primary health care delivery all over the globe (Akinyemi *et al*., 2005). Also, an appreciable number of allopathic or orthodox medicines such as quinine, morphine and acetyl salicylic acid have their sources from natural products (Akharaiyi, 2011).

Modern medications produced from plant isolates have their development based on the traditional use of the plants (Cragg and Newman, 2013). For instance, the synthesis of metformin and other bisguanidine-type anti-diabetic drugs was based on the model of galegine, a pure compound isolated from *Galega officinalis*, which is used traditionally for the treatment of diabetes (Fabricant and Farnsworth, 2001). Also, the antimalarial drug, quinine was isolated from the bark of *Cinchona officinalis*, which had long been used for the treatment of fevers (Buss *et al*., 2003). However, few plants have been scientifically assessed for their quality, efficacy and toxicity (Calixto, 2005).

Also microbial resistance to almost all existing antibiotics is on the increase (Nascimento *et al*., 2000) and there are severe side effects associated with prolong use

of synthetic drugs for treatment of diseases. An Action must be taken to address these problems.

This study therefore sought to evaluate the antimicrobial, antioxidant, antiinflammatory and wound healing properties of *H. latifolia* and *L. ovalifolia*, and their cytotoxic effects, based on the claim as wound healing agents in folklore medicine.

1.1 Antimicrobial activity

1.1.1 Plants as source of antimicrobial agents

The search for new antimicrobial agents is of great importance to scientist of this age due to the increase emergence of microbial resistance (Tenover, 2006). The antimicrobial agents search includes various sources such as the synthetic compounds as well as bioactive agents from natural products including aquatic microorganisms and medicinal plants (Agyare *et al*., 2012).

Medicinal plants are a great source of antimicrobial agents and the idea of plants been used as potential drugs for treatment of infectious diseases was well accepted even before the discovery of microbes (Rios and Recio, 2005). Antimicrobial agents that originate from plants have numerous therapeutic potential and are effective in the treatment of infectious diseases, and are devoid of the side effects that often come with the use of synthetic antibiotics. Compounds isolated from plants have been developed into various medications which have aided in the management of bacterial, fungal and other microbial infections (Agyare *et al*., 2006a).

Over the years a number of researches have been carried out to investigate the antimicrobial properties of medicinal plants and compounds isolated from these plants

all over the world. These include a research that tested 122 plant species for their antimicrobial properties in Argentina. It was reported that, *Punica granatum* and *Allium sativum* inhibited *Aspergillus niger*, *Lithrea ternifolia*, *Cassia occidentalis*, *Heimia salicifolia* and *Punica granatum* prevented the growth of *Escherichia coli*, whereas *Tabebuia impetiginosa*, *Baccharis microdonta*, *Salvia gilliesii*, *Psidium guineense* and *Rosa borboniana* inhibited *Staphylococcus aureus* (Anesini and Perez, 1993). Bakkiyaraj and Pandiyaraj (2011), reported on antimicrobial activity of *Azadirachta indica*, *Portulaca oleracea*, *Euphorbia hirta*, *Gmelina asiatica* and *Santalum album* againsts some food-borne microorganisms. Also, antimicrobial potential of *Centrosema pubescens* against a wide range of microorganisms was observed by Ekpo *et al*. (2011). Agyare *et al*. (2006b), reported the antimicrobial activity of some medicinal plants in Ghana, indicating that, methanol and petroleum ether extracts of *Nauclea latifolia*, *Bridelia atroviridis* and *Zanthoxylum gilletii* exhibited antimicrobial activity against all the test organisms,with *Pseudomonas aeruginosa* been the least susceptible to the extracts of the plants. A report by Fleischer *et al*. (2003) revealed that the leaves and seeds of *Bixa orellana* has broad spectrum antimicrobial activity.

1.1.2 Microbial resistance to antimicrobial agents

Naturally, bacteria have the genetic ability to develop resistance to therapeutic agents (Nascimento *et al*., 2000). Microorganisms that were previously known to have been controlled by antibiotics are now resistant to these same antimicrobial agents (Levy and Marshall, 2004). The problem of resistance poses great threat to public health now than ever before, because of expanding multidrug resistance in a single organism which drastically restricts therapeutic options (Levy, 2005). This has compelled a lot of big

pharmaceutical companies to leave the antibiotic discovery field to produce more profitable medications for treating other chronic disease (Projan, 2003).

Resistance to antibiotics leads to ineffective treatment of infectious diseases and increases the risk of complications resulting in fatal outcomes (Harbarth *et al.*, 2007). Inappropriate diagnosis, drug counterfeit, use of antibiotics in food production and animal rearing, non-compliance and under dosing, and sometimes uncontrolled use of antimicrobial agents are factors that have contributed immensely to the overwhelming increase in the microbial resistant menace in current years (Adu *et al*., 2014). Also the pathogenic bacteria themselves have ways and strategies of acquiring or developing resistance to antibiotics, and these include, active efflux of drugs, alteration of target sites and inactivation of antibiotics by producing enzymes that degrade them (Sibanda and Okoh, 2007).

1.1.3 Resistance modulation and antibiotic modifying activity of plants

Microbial resistance to antimicrobial agents has limited the use of known cheap but potent antibiotics (Ranjan *et al*., 2012). This has necessitated the search for new potent antimicrobial agents to combat the threat posed by resistant microbes.

However, Coates *et al*. (2002) reported the emergence of cross resistance to newly identified antibiotics and other antimicrobial agents suggesting that the new antimicrobial agents may be rendered ineffective in the very near future. Even though rational use of these medicines can decrease the rate of resistance development, it cannot eradicate the emergence of resistant strains (Sibanda and Okoh, 2007). There is therefore the need to discover and develop new compounds that will target and block resistance mechanisms to help contain and treat infections from these resistant strains (Oluwatuyi *et al*., 2004).

Research has shown that plants contain compounds with or without antimicrobial property that can cause resistant microorganisms to be susceptible to a previously ineffective antibiotic (Aiyegoro *et al*., 2009). The curative effect of plant extracts in combination with previously ineffective antibiotics has been referred to as resistance modulating activity (Gibbons, 2004).

In recent years *in vitro* antimicrobial studies conducted by researchers have shown marked increase in antimicrobial activity of some antibiotics against some resistant strains when they are combined with plant extracts. These studies include; the effect of *Acanthospermum hispidum* extract on the activities of amoxicillin and ciprofloxacin (Adu *et al*., 2011). Aiyegoro *et al.* (2009) also reported that *Helichrysum pedunculatum* leaf extracts in combination with penicillin G, amoxicillin, chloramphenicol, oxytetracycline, ampicilin, tetracycline, erythromycin and ciprofloxacin resulted in up to 60% increase in the activity of these selected antibiotics against test organisms. Also, compounds isolated from *Rosmarinus officinalis* potentiated the activity erythromycin against erythromycin effluxing strains of bacteria (Oluwatuyi *et al*., 2004).

In other studies, modification of activity of antibiotics led to nullified or reduced activity. For instance, Adu *et al*. (2014), reported that the activity of amoxicillin and ciprofloxacin was inhibited against all test organisms in the presence of *Myristica fragrance,* whereas activity of tetracycline decreased by 32 fold.

1.1.4 Methods for evaluating microbial susceptibility to antimicrobial agents and Minimum inhibitory concentration determination

Antimicrobial activity of an extract or any antimicrobial agent can be investigated using diverse methods. Nevertheless the performance of all these methods employed in determining microbial susceptibility to an agent can be judged by the minimum

inhibitory concentration (MIC), hence making the MIC a 'golden standard' considered in susceptibility testing (Andrews, 2001).

The MIC can be defined as the least concentration of an antimicrobial agent that can inhibit the growth of microorganisms (Andrews, 2001; Mazzola *et al*., 2009). It is mostly used to investigate the *in vitro* antimicrobial activity of drugs and also used as a measure to determine whether a microbial strain is resistant to an antimicrobial agent or not (Andrews, 2001).

The commonly used methods for microbial susceptibility testing and MIC determination are basically grouped into two (2), which are the diffusion and dilution methods. The diffusion method comprises of the agar well and the disc plate diffusion methods, whereas the dilution method involves the broth and the agar dilution methods.

1.1.4.1 Diffusion method

This method basically involves the diffusion of an antimicrobial agent of a known concentration from a well or a disc into an agar inoculated with a standard inoculum size of the test organism. The agent is said to exhibit an antimicrobial activity if it is able to prevent or inhibit the growth of the test microorganism, which is indicated as clear zones around the well or disc. The zone of growth inhibition gives an idea as to the efficacy of the agent on the test organism, ie the larger the zone of inhibition, the more potent the agent is against the test organism, and vice versa (Fabry *et al*., 1998). From this method, the MIC is determined by plotting a graph of diameter of mean zones of inhibition against log of concentration. The antilog of the intercept on the log of concentration axis is taken as the MIC (Wiegand *et al*., 2008).

The diffusion method can be said to be simple, reproducible and cost effective, but there are some drawbacks associated with it. The inhibition of growth by the agent in the well

or disc depend on the ability of the agent to diffuse through the solid medium (Scorzoni *et al*., 2007a; Scorzoni *et al*., 2007b), this makes it not a suitable method for the evaluation of antimicrobial property of non-polar agents and those that cannot diffuse readily through the agar. However, the potency of an antimicrobial agent is not solely dependent on its solubility and diffusion profile in the agar (Cos *et al*., 2006).

1.1.4.2 Dilution method

In this method, different concentrations of the test antimicrobial agent are prepared (through dilution) in a suitable culture medium and then inoculated with test microorganism of specific inoculum size. In this method there is direct contact of the test antimicrobial agent and organism, so there is no need for diffusion of the test antimicrobial agent into the medium. The MIC is recorded after the period of incubation as the lowest concentration of the antimicrobial agent that inhibits growth of the test organism (Cos *et al*., 2006).

Even though this method is said to be more reproducible and quantitative than the diffusion methods and is suitable for determining MIC, minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of both polar and non-polar agents, the MIC that is recorded does not always denote the exact value.

The exact or actual MIC value lies between the least concentration that inhibited growth and the highest concentration that allowed growth (Cos *et al*., 2006).

1.1.4.2.1 Agar dilution method

In the agar dilution method, a suitable solid culture medium is incorporated with antimicrobial agent at different dilutions and then inoculated with specific inoculum size of test organism. Agar is allowed to set and incubated in appropriate condition, after which growth is assessed in all the concentrations used and MIC determined. The

MIC is taken to be the least concentration of the test antimicrobial agent that did not allow growth after the period of incubation (Cos *et al*., 2006; Wiegand *et al*., 2008).

1.1.4.2.2 Broth dilution method

The broth dilution method can be of two classes, depending on volume of liquid medium employed, that is, micro-dilution or macro-dilution. Micro-dilution technique uses a little amount of the broth, between 100 and 200 µL and employs the use of micro-titre plate, while the quantity of broth employed in macro-dilution technique is between 1 and 2 mL (Wiegand *et al*., 2008; Andrews, 2001). In this method, different concentrations of the test antimicrobial agent are prepared in the liquid medium and standard inoculum size of microorganism added to it. This is followed by incubating at the required temperature and time. Turbidity, indicating microbial growth in broth is assessed at the various concentrations and MIC is determined (Wiegand *et al*., 2008; Lourens *et al*., 2004). The MIC value is recorded as lowest concentration or the highest dilution of the test agent that inhibited the growth of test organism.

Microbial growth observation can be aided by addition of tetrazolium salts or resazurin dye after the period of incubation, which causes a colour change to indicate growth or no colour change to indicate no growth (Andrews, 2001; Umeh *et al*., 2005). In the micro-dilution technique, the use of plate reader to measure absorbance can also be employed to determine the extent of microbial growth in each concentration and the MIC (Devienne and Raddi, 2002; Matsumoto *et al*., 2002). Employing the plate reader, MIC values are determined as the least concentration of test agent which completely (100%) inhibited growth of microbes (Marchal *et al*., 2013).

1.2 Free radicals and reactive oxygen species (ROS)

Free radicals are molecules that can exist independently for a brief period and are made up of one or more highly reactive unpaired electrons, which reacts with other molecule by accepting or donating electron (Cui *et al*., 2004; Valko *et al*., 2007). ROS are collective term for all forms of activated oxygen or reactive oxygen, which include free radicals like ozone (O_3) and molecular oxygen such as superoxide (O_2^-) and hydroxyl (OH^*) (Cui *et al*., 2004; Im Kim *et al*., 2010).

ROS are continuously generated in the body of humans through the process of aerobic respiration and from other exogenous sources (Narayanaswamy and Balakrishnan, 2011). These ROS when produced are acted upon and detoxified by endogenous enzyme antioxidants. However due to their high chemical reactivity, when they are generated in excess or there is inadequate antioxidants defense, they can lead to oxidative damage of biomolecules such as proteins, lipids and DNA (Cui *et al*., 2004; Farber, 1994).

Oxidative stress, that is referred to as the imbalance between the production of free radicals and opposing antioxidant defense mechanism, implicates a lot of diseases like diabetes mellitus, cancer, atherosclerosis, hypertension, ocular diseases, haematological diseases, inflammatory diseases, pulmonary diseases and neurological diseases (Cui *et al*., 2004).

1.2.1 Antioxidant activity

Antioxidants may be referred to as intracellular or extracellular substances produced to counter the activity or effects of reactive oxygen species (ROS). They are used by organisms as part of their defense mechanism against the attack of free radicals. Some benefits of antioxidants include their use as stabilizers in polymeric product,

petrochemicals, foodstuffs, pharmaceuticals and cosmetics (Pisoschi and Negulescu, 2011). Also they are used in relation to radical production and oxidative stress, cancer prophylaxis and therapy (Kalcher *et al*., 2009).

Antioxidants may be grouped into three (3) based on their mechanism of action and chemical nature. These are; enzyme, preventive and scavenging or chain-breaking antioxidants (Cui *et al*., 2004).

Enzyme antioxidants are endogenously produced to act against specific ROS and break them down to less harmful forms. Examples are superoxide dismutase (SOD) which converts superoxide radical to hydrogen peroxide, glutathione peroxidase (GPx) which detoxifies hydrogen peroxide by reducing it to water, and catalase (CAT) which also detoxifies hydrogen peroxide by decomposing it to water and oxygen (Cui *et al*., 2004).

Preventive antioxidants are extracellular and they act by binding to and sequestering molecules which promote oxidation and transition metal ions that strongly enhance formation of free radical due to the presence of unpaired electrons. Examples include transferrin and lactoferrin which bind ferric ions, ceruloplasmin (which binds Cu, catalyzes the oxidation of ferrous ions to ferric due to its ferroxidase activity and increases the binding of iron to transferrin), haptoglobins which bind hemoglobin, hemopexin which binds heme, and albumin which binds copper and heme (Cui *et al*., 2004).

The scavenging or chain-breaking antioxidants are lipid-soluble and water-soluble scavengers and they act by availing themselves for oxidation at the early stages of free radical chain reaction, thereby forming low energy products that cannot propagate the chain further. Major lipid-soluble scavengers include vitamin E (a-tocopherol),

hcarotene, and coenzyme Q (CoQ) (Murthy, 2001), whereas water-soluble scavengers consist of ascorbic acid, uric acid, and bilirubin (Frei *et al*., 1988).

1.2.2 Medicinal plants as source of antioxidants

Phenolic compounds and flavonoids widely distributed in plants have been reported to exert numerous biological activities and have also been known as the main antioxidant compounds of fruits and vegetables (Wu and Ng, 2008).

Herbs, spices and other plant material rich in phenolic compound have been extracted and are widely use in the food industries since they are able to improve the quality and nutritional value of foods by retarding oxidative degradation of lipids (Patel *et al*., 2010).

Recently, there is an increasing interest in the search for therapeutic potential of plants as antioxidants, due to adverse side effects and toxicity of existing synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Qader *et al*., 2011).

Medicinal plants containing antioxidant compounds (phenolics and flavonoids) which protects cells from the damaging effects of reactive oxygen species have already been exploited for commercial use as either antioxidant additives or as nutritional supplements, while other plant species are been investigated for their antioxidant properties. A lot of researchers have been involved in the search for new and novel antioxidants from plant source in recent years. These researches include; the antioxidant property of *Artocarpus Altilis* (Amponsah *et al*., 2014). Mensah *et al*. (2004) reported on the antioxidant activity of *Commelina diffusa*. Whereas the antioxidant properties of *Kigelia africana* and *Strophanthus hispidus* have been reported by Agyare *et al*. (2013a).

1.2.3 *In vitro* assays used to determine antioxidant activity

The *in vitro* assay methods for assessing the antioxidant activity of a product can be grouped into two (2), namely direct and indirect methods. Indirect methods do not involve oxidisable substrate and are used to measure the ability of the presumed antioxidant molecule to reduce a stable artificial free radical or a transition metal either by hydrogen or electron transfer. Example of assays that fall under indirect methods include, 2, 2- diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, total antioxidant capacity test and ferric-reducing antioxidant power assays(Laguerre *et al*., 2007).

Direct methods involve an oxidisable substrate and are used to assess the potential of the antioxidant to inhibit the oxidative degradation of the substrate in a test system subjected to natural or accelerated oxidation conditions. The oxidisable substrate normally include lipids, plant proteins, fluorophores, chromophores, DNA, fluids such as low-density lipoproteins (LDLs) and biological membranes. Example of such methods is linoleic acid autoxidation method (Laguerre *et al*., 2007).

1.2.3.1 DPPH free radical scavenging assay

The principle behind this method is based on electron transfer or hydrogen atom transfer reaction (Huang *et al*., 2005). In this method, the free radical scavenging ability of an antioxidant compound is assessed in the presence of DPPH which is a stable free radical. The DPPH which has a violet colour is reduced to a yellow chromophore in the presence of the antioxidant. After 30 min of reaction, it is expected that the supposed antioxidant substance should mop up the DPPH free radicals in solution and excess free radicals measured using spectrophotometry by taking absorbance usually at 517 nm (Prior *et al.,* 2003; Badarinath *et al.,* 2010; Khalaf *et al*., 2008).

The percentage DPPH free radical scavenging activity is calculated afterwards and used to determine the concentration that caused 50% reduction in the concentration of DPPH free radicals in solution, referred to as IC_{50} or EC_{50} (Badarinath *et al.*, 2010). This assay is very simple and rapid, but may have some few drawbacks. Compound such as carotenoids can appreciably absorb at 515 to 517nm, hence can influence spectrophotometry readings and affect the outcome of the experiment. Again, the antioxidant activity can be affected by the antioxidant's chemical structure (steric hindrance), small compounds can easily access and scavenge DPPH free radical, while bulky structures can be hindered or have difficulty in accessing free radicals, thereby appearing to have no or reduced antioxidant activity (Prior *et al.*, 2003).

1.2.3.2 Total antioxidant capacity assay

This assay involves the reduction of molybdenum by an antioxidant. Mo^{+6} is reduced to Mo^{+5} in the presence of an antioxidant compound. This reduction leads to the formation green phosphate-molybdate V (Mo^{+5}) complex at acidic pH, which is spectrophotometrically quantified by measuring absorbance at 695 nm (Prieto *et al.*, 1999). The higher the absorbance of the green phosphate-molybdate V (Mo^{+5}) complex formed, the greater the antioxidant activity of the compound and vice versa. This assay is quantitative, as it expresses the antioxidant activity as equivalence of ascorbic acid or α-tocopherol.

1.2.3.3 Total Phenol (Folin-Ciocalteu) Assay

This assay involves the reduction of phosphomolybdate phosphotungstate salts of Folin-ciocalteau by phenolic compounds in a basic medium. The reduced form of the salt gives a blue colouration and is quantified spectrophotometrically at 760 nm (Slinkard

and Singleton, 1977; Socha *et al*., 2009).This assay is also quantitative and it expresses the phenol content as tannic acid equivalent.

1.2.3.4 Ferric-reducing antioxidant power assay

This assay is based on the principle of reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) by an antioxidant compound. The resultant Fe^{2+} reacts with 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) to form a TPTZ-Fe^{2+} complex which gives an intense blue colour. Absorbance is measured at 593 nm to quantify the amount of reduced iron which is related to the antioxidant activity of the compound (Benzie and Strain, 1996; Pisoschu and Negulescu, 2011).

1.2.3.5 Lipid peroxidation inhibition assay

This assay involves peroxidation of polyunsaturated fatty acid (linoleic acid) at a temperature of 40ºC. Products formed at the primary and secondary stages of the degradation are used as a measure of the extent of lipid peroxidation, by employing ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods (Inatani *et al*., 1983).

In the ferric thiocyanate (FTC) method, peroxides produced at the primary stage of degradation oxidize Fe^{2+} (blue) to Fe^{3+} (reddish brown), which is then quantified by forming a complex with SCN^- ions (in ammonium thiocynate) and absorbance measured at 500 nm. In the presence of an agent with a high antioxidant activity, the amount of peroxides produced during peroxidation of linoleic acid is low, thereby low formation of Fe^{3+} ions and low absorbance values recorded.

In the thiobarbituric acid (TBA) method, malondialdehyde (MDA) formed by degradation of peroxides at the secondary stage is measured. Malondialdehyde (MDA) binds to thiobarbituric acid (TBA) at a low pH and high temperature (100°C) to form

TBA-MDA adduct, which gives a pink colouration that is correlated to the extent of lipid peroxidation by measuring absorbance at 532 nm. The higher the antioxidant activity, the less the intensity of the pink complex formed and hence the lower the absorbance values (Yin *et al.,* 2011; Pisoschu and Negulescu, 2011).

1.3 Inflammation

Inflammation is a complex biological mechanism employed by vascular tissue for repair when injured or when exposed to harmful stimuli like pathogens or infections, irritants and physical injuries. It is a protective action that an organism uses to remove injurious stimuli, initiate healing process, as well as generate new tissues to replace damaged ones (Singh *et al*., 2008; Schmid-Schönbein, 2006).

Classical signs associated with inflammation include, pain, redness, swelling, heat and loss of function (Shailasree *et al*., 2012; Kumar *et al*., 2010; Rock and Kono, 2008).
An organism's ability to mount an inflammatory response is very necessary for its survival when injured or when infected by pathogens and other harmful stimuli.

Inflammatory response can occur in three distinct temporal phases; (a) an acute phase which involves vasodilation and increased capillary permeability; (b) sub-acute phase, where phagocytic cell and leukocytes migrate to the inflammation site; and finally (C) chronic proliferative phase characterized by tissue degeneration and fibrosis (Burke *et al*., 2006).

The inflammatory response can only be established in the presence of inflammatory inducers and mediators. Inflammatory inducers are signals that initiate inflammation, while inflammatory mediators are substances with biological properties that cause or enhance the signs and symptoms of inflammation.

Inducers of inflammation may be endogenous and exogenous. Endogenous inflammatory inducers can be signals from stressed, malfunctioning or damaged tissues and may also include crystals of monosodium urate and calcium pyrophosphate dihydrate, advanced glycation end products (AGEs) and oxidized lipoproteins (Rock and Kono, 2008). Exogenous inducers consist of microbial and non-microbial signals. Microbial inducers can be pathogen-associated molecule patterns or virulence factors, while non-microbial inducers include allergens, irritants, foreign bodies and toxic compounds (Rizki and Rizki, 1992; Dostert *et al*., 2008).

Mediators of inflammation can be derived from plasma and blood cells or tissues. There are four major mediator-producing systems contained in plasma which interact in defined manners to generate phlogistic compounds. These include kinin, complement, coagulation and fibrinolytic systems, which are inactive proteases in their native form but are activated by proteolytic cleavage leading to the rise of more inflammatory mediators (Rang, 2007).

Cell or tissue mediators may be preformed and stored in granules or may be newly synthesized by cells. Preformed cell mediators may include histamine in mast cells and cationic proteins in neutrophils, while newly synthesized cell mediators may comprise of interleukin-1, leukotrienes, platelet-activating factor (Larsen and Henson, 1983).

Inflammation can be classified as acute or chronic, depending on the nature of the stimulus and the extent to which the stimulus was eliminated by the initial processes. Acute inflammation is an initial response of the body to harmful stimuli, which is rapid lasting for hours or few days. It can be induced by injury, infections and microbial toxins, tissue necrosis, hypoxia and immune reactions (Kumar *et al*., 2010) and is characterized by increased release of plasma and leukocytes from the blood to the

injured area (Shailasree, *et al*., 2012). This leads to the successful elimination of the harmful stimuli and complete healing of the endangered tissue. However, if the acute response is not able to eliminate the stimuli, it can result in the chronic state.

Chronic inflammation results from either the persistence of the injurious agent or some interference with the normal process of healing leading to prolonged duration of inflammation (weeks or months). It is characterized by the production of mononuclear cells including macrophages, lymphocytes and plasma cells as well as tissue destruction. Chronic inflammation may follow acute inflammation, or may begin insidiously as a low-grade smouldering response without any manifestations of an acute reaction, leading to tissue damage in most of the common and disabling human diseases like rheumatoid arthritis, atherosclerosis, tuberculosis and pulmonary fibrosis. This type of chronic inflammation may also be implicated in the progression of cancer and Alzheimer disease (Kumar *et al*., 2010).

1.3.1 Management of inflammation

Though the inflammatory response is a defensive mechanism on its own, it may lead to serious tissue damage which may call for an intervention to enhance the healing process (Obiri *et al*., 2013). This calls for the use of principal orthodox antiinflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), steroidal anti-inflammatory drugs (glucocorticoids) and disease modifying antirheumatic drugs (DMARDs). These drugs though effective, have been reported to be associated with severe side effects (Graham, 2006), which has necessitated the search for new and effective anti-inflammatory drugs with no or little adverse effects. The search is ongoing and can never be completed without investigating into natural products especially medicinal plants since they have been known as a great store of therapeutic agents (Cragg and Newman, 2013).

Medicinal plants have been promising when it comes to handling actions that are likely mediated through interference with the predominant pathophysiological processes of inflammation (Obiri *et al*., 2013). Examples of such plants with antiinflammatory activity include fruits of *Xylopia aethiopica* (Obiri and Osafo, 2013), aerial parts of *Hilleria latifolia* (Abotsi *et al*., 2012), *Ulva lactuca* (sea weed) (Margret *et al*., 2009), whole plant of *Sarcostemma secamone* (Kumari *et al* 2012), Stem bark and leaves of *Pterygota macrocarpa* and *Cola gigantea* (Agyare *et al*., 2012), leaf and bark extracts of *Funtumia elastica* (Agyare *et al*., 2013b).

1.3.2 Models used to evaluate anti-inflammatory activity of an agent

A number of animal models can be employed in both acute and chronic inflammation models to assess the anti-inflammatory activity of an extract or a compound.

1.3.2.1 Acute inflammation model

Acute inflammation may be induced by injecting various parts of an animal's body with an inflammatory agent such as killed bacteria (e.g. *Escherichia coli*), carrageenan, formalin, polymorphonuclear leucocytes, arachidonic acid (in acetone), zymosan and monosodium urate crystals (Issekutz and Issekutz, 1989; Higgs, 1989). The animal responses to such inflammatory agents and this can be monitored by increase in foot volume (oedema in rat's paw), local rise in temperature, detection of plasma markers in skin, measurement of inflammatory mediators in plasma exudates, monocytes infiltration, leucocytes and lymphocytes accumulation, and platelet deposition using various techniques (Issekutz and Issekutz, 1989).

The most widely used and acceptable preliminary animal model of acute inflammation employed in the screening of new anti-inflammatory drugs is the carrageenan-induced acute foot oedema in laboratory animals (Winter *et al*., 1962). Carrageenan which is a

polysaccharide derived from the Irish sea moss is injected into the foot of laboratory animal to induce inflammation with a characteristic swelling paw (oedema) (Morris, 2003). The animal is treated with the test drug and the volume of the oedema is assessed to ascertain the anti-inflammatory activity of the drug.

Carrageenan (phlogistic agent) is widely accepted and used in the acute inflammation model because it is known to be antigenic and is devoid of apparent systemic effect (Di Rosa, 1972; Kaur *et al*., 2004). It is also known to cause the release of more than one inflammatory mediator which is a useful tool in assessing anti-inflammatory effect of an agent. Again it has the ability to induce an intense and reproducible inflammatory action and it is sensitivity to inhibition by various anti-inflammatory drugs (Winter *et al*., 1962; Kaur *et al*., 2004).

1.3.2.2 Chronic inflammation model

A lot of models have been developed for the study of chronic inflammation. These include the polyarthritis induced by type II collagen in rats and mice (Trentham *et al*., 1977), the arthritis induced by streptococcal cell walls in the rat (Cromartie *et al*., 1977), chronic inflammation induced by implantation of cotton wool pellets subcutaneously (cotton pellet-induced granuloma) (Winter and Porter, 1957). Also, injection of turpentine oil (into pleural cavity or subcutaneous pouch) (Selye, 1953, Robert and Nezamis, 1957) and the most frequently studied model of polyarthritis induced in the rat with *Mycobacterium tuberculosis* (Freund's adjuvant arthritis) (Whicher *et al*., 1989).

Models of arthritis is employed extensively in research because of its reproducible predictiveness regarding the activity of NSAIDs, even though it is not yet useful in

finding anti-arthritic agents with disease modifying properties, and because they closely resemble chronic inflammatory disease in man (Weichman, 1989).

1.4 Wounds

Wounds are physical, chemical or thermal break in continuity of the skin or its underlying tissues leading to disturbance in the normal skin anatomy and disruption of anatomical and functional integrity of living tissues (Singh *et al*., 2006). Wounds normally serve as a passage or entry for infectious microbial agents into the inner tissues (Krasner *et al*., 2007; Ofori-Kwakye *et al*., 2011; Singh *et al*., 2006).

Wounds may be acute or chronic depending on the healing process, and open or closed based on the cause and nature of the wound.

1.4.1 Acute wounds

Acute wounds are wounds that go through the normal phases of healing thereby showing signs of healing in some few weeks. In acute wounds the processes involved in healing is always completed within the expected time frame. There is a great risk of infection within the first 72 hours of wound occurrence and for that matter proper care must be ensured within this period. Examples of acute wounds may include postoperative wounds and cuts (McGuckin *et al*., 2003).

1.4.2 Chronic wounds

Chronic wounds are those that exhibit impaired healing and have failed to go through the normal phases of healing within four weeks, thereby by showing uncoordinated, postponed and incomplete healing. That is, chronic wounds take a long time to heal, do not heal or recur more often. Examples of chronic wounds are ulcers associated with diabetes mellitus, ischemia and pressure ulcers (Guo and DiPietro, 2010; McGuckin *et al*., 2003; Krishnan, 2006).

1.4.3 Open wounds

Open wounds lead to break or crack in the skin exposing the underlying tissues to the outside environment. External bleeding is mostly observed and the risk of microbial infection is very high. Examples of open wound are punctures, lacerations, incisions, abrasions and avulsions (Lazarus *et al*., 1994; Nagori and Solanku, 2011).

1.4.3.1 Punctures

These are open wounds caused by small pointed objects such as tip of a knife, needle, a nail or a sharp tooth. Bleeding in such wounds is very minimal and are therefore at a higher risk of infection since bleeding leads to the removal of foreign materials that may enter into the depths of the wound.

1.4.3.2 Lacerations

This type of open wounds is caused by some blunt trauma resulting in tissue damage or lost. Mostly they form lines on the surface of the skin but can extend into the lower tissues.

1.4.3.3 Incisions

Incised wounds are caused by sharp-edged object such as a knife, razor or glass splinter and are characterized by no tissue loss and minimal tissue damage. Bleeding may be profuse hence immediate attention is needed.

1.4.3.4 Abrasions

Abrasions are caused when the surface of the skin gets rubbed against a rough or hard surface. Normally the topmost layer of the skin is scrapped off and the nerve endings are exposed, leading to shallow and painful injuries.

1.4.3.5 Avulsions

These are type of injury whereby a part of the body is forcefully removed from its original place. An example of this type of injury is seen in an amputation where the body part is pulled off rather than cut off. Bleeding is very severe and due to its seriousness nature, immediate attention is needed.

1.4.4. Closed wounds

These are injuries that cause damage to internal tissues and organs without any break in the outermost layer of the skin. In this type of injury, blood escapes the circulatory system but remains inside the body. The risk of microbial contamination is very minimal in closed wounds since they are not exposed to the outside environment. Examples of such wounds include bruises, haematomas and crush injury (Lazarus *et al*., 1994; Nagori and Solanku, 2011)

1.4.4.1 Haematomas

This type of closed wound is also known as blood tumor and is caused by damage to a blood vessel which results in blood been collected under the skin.

1.4.4.2 Contusion or bruises

These are injuries that cause damage to tissues under the skin and are caused by blunt force trauma.

1.4.4.3 Crush injury

Crush injuries are caused when a great or extreme amount of force is applied on the skin for a long period of time.

1.4.5 Process of wound healing

Wound healing is a complex process instigated immediately after injury to repair damaged tissues or organs. The process consist four (4) distinct but interconnected and overlapping phases which occur in proper sequence and time frame to enhance successful healing (Guo and DiPietro, 2010; Nagori and Solanku, 2011). These integrated phases are haemostasis (coagulation), inflammation, proliferation and tissue remodelling (Gosain and DiPietro, 2004).

1.4.5.1 Haemostasis phase

This phase starts right after injury or wounding resulting in vascular constriction and fibrin clot formation. Platelets aggregation at the site of injury leads to the production of thrombin and certain factors which cause the intrinsic clotting cascade to convert fibrinogen to fibrin, which forms a mesh (clot) to control active bleeding and helps to achieve haemostasis. The clot and surrounding wound tissue also lead to release of pro-inflammatory cytokines and growth factors like transforming growth factor (TGF)-β, fibroblast growth factor (FGF), epidermal growth factor (EGF) and plateletderived growth factor (PDGF) to initiate the next phase (Guo and DiPietro, 2010).

1.4.5.2 Inflammatory phase

This phase starts with the migration of pro-inflammatory cells to the wound site within 6 to 8 hours after bleeding has been controlled. The inflammatory phase is characterised by vasodilation of blood vessels and infiltration of neutrophils, which act to clear any invading microorganisms and cellular debris at the wound site. Macrophages are subsequently released to continue the degradation of microbes and other tissue debris at the wound area. Also macrophages cause the release of growth factors to enhance

healing in the proliferative phase, and lead to the multiplication of endothelial cells and smooth muscle cells. They again secrete other factors like interleukin-1 (IL-1) and tumor necrosis factor (TNF) which facilitate the healing process (Habif, 1996). Macrophages clear apoptotic cells (including neutrophils), leading to subsequent stimulation of keratinocytes, fibroblasts and angiogenesis to promote tissue regeneration (Meszaros *et al*., 2000; Mosser and Edwards, 2008).

1.4.5.3 Proliferative phase

This phase normally follows and overlaps with the inflammatory phase lasting for 2 days to 3 weeks after the inflammatory phase. At this phase fibroblasts and endothelial cells are the most prominent cells present and enhance capillary growth, collagen formation, and the formation of granulation tissue at the injury site. The proliferation phase consists of three main steps, which are granulation, contraction and epithelialization. In the granulation stage, fibroblast form collagen bed and produce new capillaries. Within the wound bed, fibroblasts produce collagen as well as glycosaminoglycans and proteoglycans which are major components of the extracellular matrix (ECM) and are essential for wound repair. At the contraction stage, pulling together of wound edges to reduce the defect is observed. During the epithelialization stage, new and fresh epithelial tissues form over the wound site, with cell migrating from the periphery of the wound and adnexal structures (Guo and DiPietro, 2010; Nagori and Solanku, 2011). Clinically, proliferation is characterized by pebbled red tissues or collagen formation in the wound base and leads to the replacement of dermal tissues and in few instances sub-dermal tissues (in deep wounds), along with wound contraction (Stadelmalmann *et al*., 1998; Kane, 2007).

1.4.5.4 Tissue remodelling phase

The remodeling phase can last for years and is generally characterized with extracellular matrix (ECM) remodelling to approach the normal or original tissue. In this phase, regression of many of the newly formed capillaries takes place, so as to cause the vascular density of the wound to return to normal, as well as physical contraction of wounds believed to be a function of contractile fibroblasts (myofibroblasts). Also intermolecular cross-linkage of collagen leads to increased tensile strength of tissue, with scar tissue becoming about 80% stronger as the original tissue (Guo and DiPietro, 2010; Nagori and Solanku, 2011).

1.4.6 Medicinal plants as source of wound healing agents

Orthodox medications have been employed to shorten the duration of the healing process and minimize complications associated with natural wound healing (such as wound infection due to bacterial contamination). However, the cost of these orthodox medications are outrageous and wound contaminating bacteria are becoming more resistant to their effect (Udegbunam *et al*., 2014). The search for newer, effective and affordable alternatives has become the focus of a lot of scientist now.

Medicinal plants have been widely used and accepted as more efficacious in traditional medicine for the treatment of wounds among past and present generations (Udegbunam *et al*., 2014). The following are some medicinal that have been reported with wound healing proprties. *Pupalia lappacea* (Apenteng *et al*., 2014; Udegbunam *et al*., 2014), *Spathodea campanulata* (Ofori-Kwakye *et al*., 2011), *Kigelia pinnata* (Sharma *et al*., 2010), *Carica papaya* (Mahmood *et al*., 2005), *Myrianthus arboreus* and *Alchornea cordifolia* (Agyare *et al*., 2014), *Rubus sanctus* (Süntar *et al*., 2009), *Aloe vera* (Yadav *et al*., 2012; Oryan *et al*., 2010), *Justicia flava, Lannea welwitschii*

(Agyare *et al*., 2013c), *Strophanthus hispidus* (Agyare *et al*., 2013a) and *Clerodendron slendens* (Gbedema *et al*., 2010).

1.4.7 Models for assessing wound healing activity of an agent

A lot of models have been developed to evaluate the influence of an agent on the healing processes and these models can be classified as *in vitro* and *in vivo*. For any of the models to be employed in a study, a number of factors must be considered, especially the parameters to be measured.

1.4.7.1 *In vitro* wound healing models

The *in vitro* models involve minimal ethical consideration, are very rapid and simple, and are cost effective as compared to the *in vivo* models. The *in vitro* models available for assessing the wound healing activity of an agent include single cell systems, multicellular systems and organ cultures (intact skin). These models can be employed in a study to mimic cell migration and proliferation, cell-cell and Cell-matrix interaction, protein synthesis, wound contraction and epithelialisation during wound healing (Gottrup *et al*., 2000).

1.4.7.2 *In vivo* wound healing models

In vivo models employed in the study of the wound healing activity of an agent can grouped into artificial and tissue models. Artificial models consist of subcutaneous chamber or sponges and subcutaneous tubes, while tissue models include excisional wounds, incisional wounds, superficial wounds, dead space and burn wounds (Dorsett-Martin and Wysocki, 2008; Gal *et al*., 2008).

Moreover models such as rabbit ear chamber, the hamster check pouch, the rabbit corneal pocket and the chick chorioallantoic membrane can be employed to assess

parameters such as epithiliasation, neovascularization and dermal reconstitution (Gottrup *et al*., 2000).

1.5 In vitro cell toxicity studies

The possible side effects and toxicity of medicinal plant and other compounds used for the treatment of diseases is an important part of new drug discovery and these cannot be overlooked. This has led to the development of various cytotoxicity assays including 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay to measure cell viability, 5-bromo-2-deoxyuridine (BrdU) assay to measure cell proliferation and Lactate dehydrogenase (LDH) release assay to assess the integrity of the cell membrane (Döll-Boscardin *et al*., 2012).

1.6 Plants under study

1.6.1 *Hilleria latifolia* (Lam.) H. Walt.

Description

Hilleria latifolia (Lam.) H. Walt belongs to the family Phytolaccaceae and is locally known as '*Avegboma'*, '*Boe'* or '*Kukluigbe'* by the Ewes and '*Anafranaku'* by the Asantes. It is a perennial herb of 30 to 120 cm high. The leaves are ovate-elliptic, 15 cm long and 6 cm broad, obtuse or sub-acute at the base, with numerous short hairlike structures on lower surface. Petiole is 3 to 7 cm long whilst flowers are pink or white in slender racemes up to 13 cm long. The fruits are reticulate, glabrous and ellipsoid-globose

(Mshana, 2000; Dokosi, 1998).

Figure 1.1: Aerial parts of *Hilleria latifolia* **Geographical distribution**

H. latifolia is common on cultivated grounds and along forest paths in Ghana. It also occurs in other parts of tropical Africa as well as South America. It can also be found along the West African cost, from Sierra Leone to Cameroun (Dokosi, 1998).

Medicinal uses

The leaves are used for the management of rheumatism, boils and wounds in Ghana (Agyare *et al*., 2009). The leaves, added to those of *Piper guineense*, are applied to the body for the treatment of general oedema. In Congo, the leaves are employed to treat some skin diseases (Mshana, 2000; Dokosi, 1998).

Leaf decoction is taken or administered by enema to treat ascites and food poisoning as it causes violent purging. Also it is taken to treat coughing of blood (Schmelzer and Gurib-Fakim, 2008). A paste made from grounded flowers is taken together with fresh orange juice in the treatment of asthma (Mshana, 2000).

Other uses

H. latifolia is used as an indicator of good soil and climate condition for the cultivation of cocoa and coffee. The dried seeds are used as a relish to tortoise meat (Dokosi, 1998).

The blackened stem is used by the Maasai people of Kenya in drawing eyebrows (Schmelzer and Gurib-Fakim, 2008).

Phytochemical constituents

Preliminary phytochemical screening of the ethanolic extract of the aerial parts of *H. latifolia* showed the presence of saponins, tannins, glycosides, steroids, terpenoids as well as small amounts of flavonoids and alkaloids (Woode *et al*., 2011). In another report, methanolic fraction of *H. latifolia* showed the presence of glycosides, coumarins and reducing sugars, as well as small amount of triterpens and sterols.

However, saponins, tannins, flavonoids and alkaloids were absent (Assob *et al*., 2011).

Biological activity

The leaf extract of *H. latifolia* has been demonstrated to have anxiolytic and antidepressant-like effects, antimicrobial, antioxidant and anti-inflammatory properties, (Abotsi *et al*., 2012; Assob *et al*., 2011; Woode *et al*., 2011), antinociceptive and some neurobehavioral properties (Woode and Abotsi, 2011).

1.6.2 *Laportea ovalifolia* (Schumach.) Chew.

Description

Laportea ovalifolia (Schumach.) Chew belongs to the family Urticaceae. It is known by the Asantes as 'akyekyenwonsa', 'abrewa nom taa' or 'Kumasi otuo'. It is a herbaceous weed more often creeping than erect, densely covered with stinging hairs. The perennial stems are cylindrical, greenish to sometimes reddish or brownish in colour often prostrate with erect shoots (Chew, 1969). *L. ovalifolia* is of two varieties that is *L. ovalifolia* (male and female). Since they are related species, they have the same characters but different in structure (Yang *et al*., 1996). *L. ovalifolia* (male) have big leaves and (female) possess small leaves (Essiett, *et al*., 2011).

Figure 1.2: Leaves of *Laportea ovalifoila*

Geographical distribution

L. ovalifolia is a tropical plant commonly found in swampy areas in Cameroon and other parts of the world in both dry and rainy seasons (Letouzey, 1968).

Medicinal uses

The leaves of *L. ovalifolia* are used as haemostatic on cuts and wounds (Agyare *et al*., 2009), whereas the fruits are used as a poison antidote (Bouch, 2004). The root boiled in water is taken to prevent excessive menstrual bleeding (Sofowora, 1996).

Other uses

The people of Ibibio tribe in Nigeria use the leaves and tender shoots of the plant as pot herb or vegetables in soups (Etukudo, 2003)

Phytochemical constituents of the plant

Phytochemical analysis of the leaves extract of *L. ovalifolia* revealed the presence of saponins, tannins, flavonoids, phlobatanins and cardiac glycosides. Anthraquinone was absent (Essiett, *et al*., 2011).

Biological activity

L. ovalifolia has been reported to possess antimicrobial, antihyperglycemic activity and it is also effective in reducing oxidative stress in diabetes (Okwulehie and Akanwa, 2013; Iffen and Usoro, 2010). It also has antidiabetic and hypolipidemic effects in alloxan induced diabetic rats (Momo *et al*., 2006).

1.7 Main objective

The aim of this study was to determine the antimicrobial and resistance modifying activities, anti-inflammatory and *in vivo* wound healing activities of leaf and root methanol extracts of *H. latifolia* and leaf methanol extract of *L. ovalifolia*.

1.7.1 Specific objectives

- To prepare leaf and root methanol extracts from *H. latifolia* and leaf extract of *L. ovalifolia*.
- To determine the phytochemical constituents and HPLC profile of the extracts.
- To determine the antimicrobial properties and minimum inhibitory concentrations of the extracts.
- To determine the ability of the extracts to modulate the resistance of some organisms to selected antibiotics.
- To determine the antioxidant properties of the extracts.
- To determine the anti-inflammatory properties of the extracts.
- To determine the wound healing potential of the extracts.
- To evaluate the cytotoxicity effect of the extracts

CHAPTER TWO

2.0 Materials and methods

2.1 Materials

Table 2.1.1 List of instruments and equipment

Instruments/Equipment/Materials	Manufacturer/source/city
Lab mill machine	Christy and Norris, England
Ultra-Turrax (T 25)	Janke and Kunkel, Labortenik, Germany
laboratory sieve	Retsch, Haan, Germany
Filter paper	Whatmann, UK
Rotary evaporator (R-210)	Buch, Germany
Hot air oven	Gallenkamp, UK
Refrigerator	Sharp Corporation, UK
Beakers	Fisher Scientific, Schwerte, Germany
Conical flask	Fisher Scientific, Schwerte, Germany
Test tubes	Fisher Scientific, Schwerte, Germany
Electronic weighing balance	Ohaus Corporation, PB, NJ, USA
Thermostatically controlled water bath	New Brunswick, Edison, N J, USA
HPLC machine	Agilent, USA
HPLC column	Phenomenex Jupitoer C18 300R 250 x 4.6 mm 5 microns
Syringe filters (0.45 µm pore size)	Sartorius Stedim Biotech, Goettingen, Germany
Frost free freezer	Mistral, UK
Micropipettes	Fisher Scientific, Schwerte, Germany
Pipette tips (10, 100, 1000 µL)	Sarstedt, Nümbrecht, Germany
96 well microtitre plates	Sarstedt, Nümbrecht, Germany
Laminar air flow cabinet Model T 22472	Skan AG, Allschwill, Switzerland
Portable autoclave	Basildon Ltd.UK
Incubator	Thermo Scientific, Asheville, NC, USA
Microtitre Plate Reader (MTP-Reader)	Bio Tek Instruments GmbH, Germany
Electronic calipers (Z22855,	Milomex Ltd, Bedfordshire, UK).
Surgical scissors	J J Int. Instruments, Kerala, India
Toothed forceps	J J Int. Instruments, Kerala, India
Axioscop Zeiss Microscope	Carl Zeiss Microscopy, Thornwood, USA
Cell Proliferation ELISA, (5-bromo-2-deoxyridine, BrdU) Test Kit	Roche Diagnostic GmbH, Mannheim, Germany
Cytotoxicity Detection Kit (LDH), Colorimetric	Roche Diagnostics GmbH, Mannheim, Germany

Table 2.1.2 List of culture media

Culture medium	Manufacturer/Company/Place
Mannitol salt agar	Oxoid Ltd, Basingstoke, UK
Cetrimide agar	Oxoid Ltd, Basingstoke, UK
MacConkey agar	Oxoid Ltd, Basingstoke, UK
Sabouraud agar	Oxoid Ltd, Basingstoke, UK
Sabouraud broth	Oxoid Ltd, Basingstoke, UK
Nutrient agar	Oxoid Ltd, Basingstoke, UK
Nutrient broth	Oxoid Ltd, Basingstoke, UK
Bismuth sulphite agar	Oxoid Ltd, Basingstoke, UK
Koser's citrate	Oxoid Ltd, Basingstoke, UK
Methyl Red Voges Proskauer (MRVP) broth	Oxoid Ltd, Basingstoke, UK

Tryptone broth	Oxoid Ltd, Basingstoke, UK
Peptone broth	Oxoid Ltd, Basingstoke, UK
HaCaT-keratinocyte medium	PAA Laboratories Pasching, Austria

Table 2.1.3 List of chemicals/ reagents and reference drugs

Chemicals/Reagents/Reference drugs	Manufacturer/source/city
Methanol	Merck BDH, Poole, UK
Ferric chloride (99.9%)	Sigma Aldrich, London, UK
Lead acetate	Sigma Aldrich, London, UK
Ammonia (99.9%)	Sigma Aldrich, London, UK
Hydrochloric acid (36.5%)	Sigma Aldrich, Michigan, USA
Sulphuric acid (98%)	Sigma Aldrich, London, UK
Sodium hydroxide (97%)	Sigma Aldrich, London, UK
Fehling's solution	GPR, BDH, Poole, UK
Ethanol	Merck BDH, Poole, UK
Chloroform (99.9%)	Merck BDH, Poole, UK
Dragendorff's reagent (99.8%)	Sigma Aldrich, London, UK
Acetic anhydride (99.9%)	Merck BDH, Poole, UK
Glycerol (88%)	Merck BDH, Poole, UK
Ciprofloxacin (98% HPLC grade)	Sigma Aldrich, Michigan, USA
Ketoconazole (98% HPLC grade)	Sigma Aldrich, Michigan, USA
Amoxicillin	Sigma Aldrich, Michigan, USA
Erythromycin	Sigma Aldrich, Michigan, USA
Tetracycline	Sigma Aldrich, Michigan, USA
Ampicillin	Sigma Aldrich, Michigan, USA
3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) (97%)	Sigma Aldrich, Taufkirchen, Germany
1, 1-diphenyl-2-picryl-hydrazyl (DPPH) (98%)	Sigma Aldrich, Taufkirchen, Germany
α-tocopherol (95.5%)	Sigma Aldrich, Taufkirchen, Germany
Tannic acid (98%)	Merck BDH, Poole, UK
Folin Ciocalteau (2 M)	Sigma Aldrich, St. Louis, Missouri, USA
Sodium carbonate (99.9%)	Sigma Aldrich, St.Louis,Missouri, USA
Ammonium molybdate (99.9%)	Sigma Aldrich, St. Louis, Missouri, USA
Disodium phosphate (99.9%)	Sigma Aldrich, St. Louis, Missouri, USA
Lambda Carrageenan	Sigma Aldrich, St. Louis, Missouri, USA
Aspirin (99% w/w HPLC)	Sigma Aldrich, St. Louis, Missouri, USA
Ketamine hydrochloride	Pfizer, New York, USA
Normal saline (0.9%)	Intravenous infusions, Koforidua, Ghana
Silver sulphadiazine cream (1% w/w)	Ayrton drugs, Accra, Ghana
Buffered Formalin (10%)	Sigma Aldrich, Michigan, USA
Xylene (99%)	Merck, BDH Poole, UK
Paraffin	Paraplast Plus, Sherwood, St. Louis, USA
Haematoxylin (90%)	Sigma Aldrich, St. Louis, Missouri, USA
Eosin (99%)	Sigma Aldrich, St. Louis, Missouri, USA
Phosphate buffer	Sigma Aldrich, St. Louis, Missouri, USA
Dimethyl sulfoxide (DMSO)	Applied Chem GmbH, Darmstadt, Germany
Foetal calf serum (FCS)	Invitrogen, Karlsruhe, Germany
Triton-X 100	Sigma Aldrich, St. Louis, Missouri, USA

2.2 Methods

2.2.1 Plant collection and extraction

Plant parts of *H. latifolia* (leave and roots) and *L. ovalifolia* (leaves) were collected from Aburi in the Eastern region of Ghana on February, 2014. The plants were authenticated by Dr. Alex Asase of the Department of Botany, University of Ghana, and voucher specimen AA 63 and AA 71, respectively deposited in the Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra. The plant parts collected were washed thoroughly under running tap-water and dried under shade at a temperature of 25 to 28^{o}C for two weeks, after which they were pulverized into coarse powder using the laboratory milling machine.

Eight hundred grams (800 g) each of the powdered plant materials were soaked or suspended in 2.5 L of the 70% v/v methanol. They were extracted with ultra-turrax under ice-cooling at a speed of 24000 rpm for 3 to 5 min**,** and then filtered using a laboratory sieve of mesh number 200 with aperture of 75 μm and Whatmann filter paper No.1. The filtrates were concentrated with the rotary evaporator at 40$_{o}$C under reduced pressure and allowed to dry in the hot air oven at 40$_{o}$C and then stored in air tight containers at 4-8$_{o}$C in a refrigerator. The weight of the extracts were recorded and labeled as *Hilleria latifolia* leaf methanol extract (HLML), *Hilleria latifolia* root methanol extract (HLMR) and *Laportea ovalifolia* leaf methanol extract (LOML).

2.2.2 Preliminary phytochemical screening

HLML, HLMR and LOML and the powdered plant materials were subjected to qualitative phytochemical screening to identify the various secondary metabolites present, using the methods described by Sofowora, (1993), Trease and Evans (2002), Ayoola *et al.* (2008), Vaghasiya *et al*. (2011) and Usman *et al.* (2014).

2.2.2.1 Test for Tannins

Twenty-five milliliters (25 mL) of water was added to 0.5 g of each of the powdered plant materials, HLML, HLMR and LOML and then boiled for 5 minutes. The solution was allowed to cool and filtered. Ten (10 mL) of water was added to 1 mL of the filtrate, followed by the addition of 5 drops of 1% lead acetate solution. The appearance of buff precipitate indicated the presence of tannins. The method was repeated using 1% w/v ferric chloride ($FeCl_3$) solution and observed for the appearance of dark green or blue-black colouration indicating the presence of condensed or hydrolysable tannins respectively.

2.2.2.2 Test for flavonoids

An amount of 0.5g each of the powdered plant materials, HLML, HLMR and LOML was separately dissolved in distilled water and filtered. A strip of filter paper was dipped in the liquid extracts and allowed to dry and then exposed to ammonia solution. The formation of an intense yellow colour which turned colourless after 5 min exposure to hydrochloric acid indicates the presence of flavonoids.

2.2.2.3 Test for glycosides (general test)

An amount of 200 mg each of the dried powdered plant materials, HLML, HLMR and LOML was warmed with 5mL of dilute H_2SO_4 for 2 minutes. Each solution was cooled and filtered. The filtrates were made alkaline by adding 2 to 5 drops of 20% NaOH, (red litmus paper turned blue), after which 1 mL of Fehling's solution A and B was added and heated on water bath for 2 minutes. The mixture was observed for the formation of a brick red precipitate which indicates the presence glycosides.

2.2.2.4 Test for saponin glycosides

Fifty milligrams each of the powdered plant materials, HLML, HLMR and LOML was shaken with 5 mL distilled water and filtered into a test tube. The filtrate was shaken vigorously to produce a froth which does not break readily on standing. The persistence of the froth for about 10 minutes after addition of 0.5 mL 2 M HCL is an indication for the presence of saponins.

2.2.2.5 Test for alkaloids

Fifty milligrams each of the powdered plant materials, HLML, HLMR and LOML was extracted with ammoniacal alcohol (95% ethanol: ammonia 9:1), filtered and evaporated to dryness. The residue was extracted with 1% H_2SO_4, filtered and rendered distinctly alkaline with dilute NH_3 solution. Afterwards it was shaken with $CHCl_3$ and the chloroformic extract layer was separated and evaporated to get rid of the $CHCl_3$. The residue was then dissolved in 1% H_2SO_4 and 1 to 2 drops of Dragendorff's reagent was added. An orange red precipitate produced is an indication of the presence of alkaloids.

2.2.2.6 Test for sterols

Fifty milligrams each of the powdered plant materials, HLML, HLMR and LOML was extracted with chloroform and filtered into tests tubes. Two (2) mL acetic anhydride was added to 2 mL of the chloroformic extract and then concentrated H_2SO_4 was carefully added at the side of the test tube. An appearance of a bluishgreen colour at the interface is an indication of the presence of sterols.

2.2.2.7 Test Terpenoids

Fifty milligrams each of the powdered plant materials, HLML, HLMR and LOML was extracted with chloroform and filtered, followed by careful addition of concentrated H_2SO_4. A reddish-brown colouration at interface indicates the presence of terpenoids.

2.2.3 High performance liquid chromatography (HPLC) Profile of extracts

The HPLC analysis was performed to know the chemical profiling of the crude extracts, which will serve as a guide in identifying the plants. The profiles of the extracts were determined using an HPLC machine with a UV-detector set at a wavelength of 254 nm. The extract was first dissolved in methanol and filtered through a syringe membrane filter (pore size 0.45 μm) into a vial and then inserted into HPLC. The running conditions included injection volume of 10 μL, mobile phase of methanol:water (20:80 v/v), flow rate of 1 mL/min and pressure of 15 MPa.

All the chromatographic data were determined using Chrom Quest software.

2.2.4 Test microorganisms

The microorganisms that were used in all the antimicrobial studies were obtained from the microbiology laboratory of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. They included, *Pseudomonas aeruginosa* ATCC 4853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 10073, *Streptococcus pyogenes* (clinical strain), *Candida albicans* (clinical strain) and *Klebsiella pneumonia* (clinical strain). The organisms were cultivated on their respective selective media and other biochemical tests were performed on them to confirm their authenticity. They were stored in 30% glycerol

broth at - 4^0C in a frost free freezer until needed, whereby 100µL of the stock suspension was transferred into broth and incubated at 37^0C for 24 h (sub-cultured) before use.

2.2.5 Determination of antimicrobial activity

2.2.5.1 Determination of MIC of extracts

The susceptibility and minimum inhibitory concentration (MIC) of HLML, HLMR and LOML were determined against the test microorganisms using the broth dilution method as described by Eloff (1998) and Agyare *et al.* (2012). Each well of the micro-titre plate (96 wells) was filled with 100 µL of double strength nutrient broth,

20 µL of 10^6 cfu/mL of the test organisms and 80 µL of different concentrations of HLML, HLMR and LOML and reference drugs prepared with sterile distilled water to obtain a gradient concentration ranging from 1.56 to 100 mg/mL and 1.0 to 128 µg/mL, respectively. Control wells were filled with broth only and broth and test organisms only. After 24 hours of incubation, 20 µL of 1.25 mg/mL of 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was added to each well and observed for a purple colouration after incubating at 37ºC for 30 min which indicated microbial growth. The least concentration of HLML, HLMR, LOML and reference drugs that did not show any colour change in the wells was recorded as the MIC.

2.2.5.2 Antibiotic modifying activity

This assay was performed to determine the effect of a sub-inhibitory concentration of the extracts on the activity of some selected antibiotics. The broth dilution assay with few modifications as described by Adu *et al.* (2014) was employed. The antibiotics used included amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin.

The MICs of the antibiotics were first determined using concentrations ranging from 1 to 1024 µg/mL. Each of the 96 wells of the micro-titre plate was filled with 100 µL of double strength nutrient broth, appropriate volume of the antibiotics and 20 µL of 10^6 cfu/mL of the test organisms. The plate was incubated for 24 h at 37°C, after which 20 µL MTT was added to the wells and MIC determined as the lowest concentration at which no growth was observed, (that is no colour change from yellow to purple).

The MICs of the antibiotics were re-determined in the presence of sub-inhibitory concentration (5 mg/mL) of the extracts (HLML and LOML).

2.2.6 Determination of antioxidant activity

2.2.6.1 DPPH free radical scavenging assay

This method was employed to assess the ability of HLML, HLMR and LOML to scavenge the DPPH free radical. Different concentrations (15.6 to 1000 µg/mL) of HLML, HLMR and LOML and standard antioxidant (α-tocopherol) were prepared in methanol. DPPH solution (20 µg/mL) was prepared with methanol in the dark. Three milliliters (3 mL) of DPPH solution was added to 1 mL each of HLML, HLMR, LOML and α-tocopherol and incubated in dark for 30 mins. Control was prepared by adding 3 mL of DPPH solution to 1 mL methanol and treated in the same way as the test samples. Absorbance of excess DPPH was measured at a wavelength of 517 nm (Susanti *et al*., 2007; Braca *et al*., 2001). The experiment was performed in triplicates.

The percentage scavenging was calculated using the equation below:

$$\% \text{ scavenging} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}})/ \text{Absorbance}_{\text{control}}] \times 100$$

2.2.6.2 Total phenolic content

The total phenolic content of HLML, HLMR and LOML were determined by the method described by Škerget *et al*. (2005), using Folin-Ciocalteu reagent with few modifications. HLML, HLMR, LOML and standard drug (tannic acid) with concentration range of 0.5 to 10 mg/mL and 0.0156 to 1 mg/mL, respectively, were prepared. A volume of 0.1 mL Folin-Ceucalteu reagent was added to 0.5 mL each of HLML, HLMR, LOML and tannic acid solution, followed by the addition of 2.5 mL sodium carbonate (2%) and incubated at room temperature for 20 min. Absorbance was taken at 760 nm. The experiment was performed in triplicates. A blank was prepared by adding all the reagents with the exception of extract/standard drug and treated likewise. The total phenolics were expressed as milligrams per gams of tannic acid equivalents (TAEs).

2.2.6.3 Total antioxidant capacity (Phosphomolybdenum antioxidant assay)

The method described by Prieto *et al*. (1999) was used to determine the ability of HLML, HLMR and LOML to reduce Mo-VI to Mo-V with subsequent formation of green phosphate-molybdate complex in an acidic pH condition. HLML, HLMR, LOML and standard drug (α-tocopherol) concentrations ranging from 0.5 to 10 mg/mL and 0.008 to 0.03 mg/mL respectively were prepared. To 1 mL each of HLML, HLMR, LOML and standard drug, 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) was added and incubated at 95^0C for 90 mins. A blank (all reagents without extract/standard drug) was treated in the same manner. The mixture was allowed to cool and absorbance was measured at 695 nm.

2.2.7 Experimental animals

Sprague-Dawley rats (150 to 200g) were obtained from the Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana animal house and kept in stainless steel cages with soft wood shavings as bedding. They were maintained under standard environmental conditions of temperature (30±2°C) and adequate humidity, with a twelve hour cycle of light and darkness. The animals were fed with standard pellet diet and provided with water *ad libitum*.

2.2.8 Ethical clearance

The experiments were conducted in accordance with accepted principles for laboratory animal use and care (EU directive of 1986:86/609/EEC) and approval from the Animal Ethical Committee, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

2.2.9 Determination of acute anti-inflammatory activity of extracts

The carrageenan induced foot oedema model in rats as described by Winter *et al*. (1962) was employed to assess the extracts ability to inhibit/reduce the paw oedema or swelling. Two experiments were performed to assess the acute anti-inflammatory activity of HLML, HLMR, and LOML. The first was to determine the effect of the drugs given 1 h before inducing the oedema (prophylaxis), and the second was to examine the therapeutic effect of the drug, whereby the drug was administered 1 h post oedema induction.

Sprague-Dawley rats were weighed and assigned randomly into groups consisting of four rats each. The initial foot volumes of the rats were measured using an electronic caliper after which reference drug (100 mg/kg aspirin) and extracts (30, 100 and 300

mg/kg), prepared in sterile distilled water, administered orally to the respective groups, with the control group given 0.5 mL sterile distilled water. After an hour of administering the drugs, oedema was induced by injecting 0.1 ml of 1% w/v carrageenan solution (in sterile distilled water) into the sub-plantar tissue of the right foot pads of the rats. Foot volumes were again measured at an hourly interval for 6 hours.

In the therapeutic experimental protocol, eodema was induced and measured 1h before administering the extracts and reference drug.

Inhibition of inflammation was calculated using the relation;

$$\% \text{ change in paw thickness} = 100 \text{ x } \left[\frac{(T_f - T_i)}{T_i}\right]$$

Where

T_i is paw thickness before carrageenan injection

T_f is paw thickness at time T.

Raw scores for right foot thickness were individually normalised as percentage of change from their values at time 0 and then averaged. Total pedal oedema was calculated in arbitrary units as the area under the curve (AUC) and to determine the percentage inhibition of oedema, the following equation was used:

$$\% \text{ inhibition of oedema} = \left[\frac{AUC_{(control)} - AUC_{(treatment)}}{AUC_{(control)}}\right] \text{x } 100$$

2.2.10 Wound healing activity of extracts

The ability of HLML, HLMR and LOML to promote wound healing *in vivo* was assessed using the excision wound healing model.

2.2.10.1 Formulation of extracts into cream

Aqueous cream base was prepared following the protocol described in the British Pharmacopoeia (2000), without the preservative (phenoxyethanol). HLML, HLMR and LOML were incorporated into the aqueous cream to give different concentrations of 5 and 10% w/w. The formulated creams were stored in the refrigerator at 25ºC and constantly monitored for physical changes like phase separation, colour, odour, and texture.

2.2.10.2 Excision wound model

The excision wound model described by Morton and Malone (1972) and modified by Agyare *et al.* (2013c) was employed in this study. The dorsal fur of rats was completely removed using new and clean razor blades. The area that was shaved was neatly wiped with 70% v/v ethanol and the rats were anaesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg body weight). Sterile surgical scissors and toothed forceps were used to make full thickness wounds of about 20 to 25 mm diameter at the back of the rats.

The rats were randomly selected into different groups with five (5) rats in each group. Each group received a respective treatment as follows:

Group A: No treatment

Group B: Treated with aqueous cream base

Group C: Treated with silver sulphadiazine (1% w/w)

Group D and E: Received 5 and 10% w/w HLML aqueous creams, respectively. Group F and G: Received 5 and 10% w/w HLMR aqueous creams, respectively.

Group H and I: Received 5 and 10% w/w LOML aqueous creams, respectively.

2.2.10.3 Treatment of wounds and wound diameter assessment

Wounds were not treated for the first 24 h period, after which they were treated daily for fifteen (15) days by topical application of 0.1 g of the respective creams. All the wounds were cleansed with normal saline (0.9% w/v) before treatment.

Diameter of the excised wounds was measured with the aid of a pair of divider and meter rule on every other day to assess the rate of wound contraction. Percentage wound closure was calculated as a percentage of the original wound from day 1 post wounding and result expressed as mean ± standard error mean (SEM). The equation below was used to calculate the percentage wound closure:

$$\% \text{ wound closure} = [(\text{wound size}_{\text{initial}} - \text{wound size}_{\text{final}}) / \text{wound size}_{\text{initial}}] \times 100$$

2.2.10.4 Histological studies

Treated and untreated wound tissues from all the groups were excised and kept in 10% neutral buffered formalin on the 15th day post wounding for histological examination to assess the degree of tissue repair (re-epithelisation, angiogenesis, collagenation and granular tissue formation). The tissues stored in buffered formalin were dehydrated several times in different concentrations of ethanol (50%, 70%, 95% and 100%), after which they were rinsed a number of times in xylene to remove ethanol and then embedded in paraffin to strengthen them for easy dissection. These tissues were sectioned into a thickness of about 5 μm and later 'deparaffinised'. The tissues were then mounted on a clean glass slides and stained with haematoxylin and eosin, they were finally observed under the microscope (Udupa *et al*., 1995; Chung *et al.*, 2010; Talekar *et al*., 2012).

2.2.11 *In vitro* cell toxicity studies of extracts

The effect of the extracts on cell viability and LDH release was investigated using HaCaT-keratinocytes (skin cell line) which was kindly provided by Professor N. E. Fusenig, German Cancer Research Center (Deutsche Krebsforschungszentrum) in Heidelberg, Germany.

2.2.11.1 Determination of the influence of HLML, HLMR and LOML on cell viability

The method as described by Mosmann (1983) and modified by Agyare *et al*. (2011) was employed to assess the effect of HLML, HLMR and LOML on cell viability.

Different concentrations of HLML, HLMR, and LOML (0.1, 1.0, 10.0 and 100.0 µg/mL) were prepared in HaCaT keratinocytes medium in 96-well micro-titre plate and seeded with 10^5 cells per well. The plates were then incubated in 5% CO_2 at 35ºC after which the medium was removed and cells washed with 100 µL of phosphate buffer solution (PBS). This was followed by the addition of 50 µL MTT solution and re-incubated to allow the formation of insoluble formazan crystals, which were then dissolved in 50 µL of DMSO to give a violet colour. The intensity of the violet coloured dissolved formazan crystals was measured by taking absorbance at 595 nm against 690nm, which correlates with the number of viable cells. HaCaT keratinocyte cells treated with 1% foetal calf serum (FCS) and untreated cells served as positive and negative controls respectively.

2.2.11.2 LDH cell toxicity assay

HaCaT keratinocytes (10^5 cells per well) were incubated at 35ºC for 24 h in the presence of different concentrations (0.1, 1.0, 10.0 and 100.0 µg/mL) of HLML, HLMR and LOML. Twenty-five micro-litres of the reaction mixture (Lactate, NAD+, INT and diaphorase) was then added and incubated in the dark at 20ºC for 30 min. The reaction was terminated by the addition of 10 µL 1M HCL solution to each well. The absorbance

of the resultant solutions was measured with MTP reader at 490 against 690 nm. Untreated cells and cells treated with lysis solution (10% Triton X100 in 5% FCS) were used as negative and positive controls respectively. (Decker and Lohmann-Matthes, 1988)

2.3 Statistical analysis

Data were presented as the mean ± standard error mean (SEM) in the studies. Analysis of results was done using one-way and two-way ANOVA followed by the Dunnett's/Tukey's and Bonferroni's *post hoc* test respectively. Graphs were plotted with Graph Pad Prism for windows version 6 (Graph Pad, San Diego, CA, USA).

CHAPTER THREE

3.0 RESULTS

3.1 Plant extraction

Extraction of medicinal plant parts with a selective solvent is necessary to attain desired therapeutic portions and to exclude unwanted material (Tiwari *et al*., 2011). The maceration extraction procedure is highly accepted since most plant constituents may be thermolabile (Ncube *et al*., 2008). The quantity of the 70% methanol extract obtained after cold maceration was expressed as percentage in relation to the quantity of the dried powdered plant materials used for the extraction, to give the percentage yield. The percentage yield for HLML, HLMR and LOML were 17.49, 7.50 and 11.29%, respectively (Table 3.1.1).

Table 3.1.1 Percentage yield of methanol extracts of *H. latifolia* and *L. ovalifolia*

Code	Extracts	% yield
HLML	*Hilleria latifolia* leaf methanol extract	17.49
HLMR	*Hilleria latifolia* root methanol extract	7.50
LOML	*Laportea ovalifolia* leaf methanol extract	11.29

3.2 Preliminary phytochemical screening

Phytochemical screening has been used over the years to detect diverse groups of naturally occurring plant metabolites and is considered as an effective approach for discovering bioactive profile of plants of therapeutic importance (Masih and Singh, 2012). Various phytochemical tests were performed on HLML, HLMR and LOML and their dried powdered plant materials, respectively to identify their phytochemical composition.

Phytochemical screening of HLML and the dried powdered leaf material of *H. latifolia* revealed the presence of tannins, flavonoids, glycosides, saponins, alkaloids, sterols and terpenoids. In HLMR and the dried powdered root material of *H. latifolia*, tannins, glycosides, saponins, alkaloids, sterols and terpenoids were present whereas flavonoids were absent. LOML and the pulverized leaf material of *L. ovalifolia* also showed the presence of tannins, glycosides, sterols and terpenoids. Additionally, saponins were also found in the pulverized leaf material of *L. ovalifolia* (Table 3.2.1).

Table 3.2.1: Phytochemical screening of HLML, HLMR, LOML and their dried powdered plant samples

Secondary metabolites	*H. latifolia* leaf		*L. ovalifolia* leaf		*H. latifolia* root	
	HLML	Powdered plant material	LOML	Powdered plant material	HLMR	Powdered plant material
Tannins	+	+	+	+	+	+
Flavonoids	+	+	-	-	-	-
Glycosides	+	+	+	+	+	+
Saponin	+	+	-	+	+	+
Alkaloids	+	+	-	-	+	+
Sterols	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+

(+) = presence of secondary metabolites; (-) = absence of secondary metabolites. HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract.

3.3 HPLC Profiles of extracts

The HPLC profiles of HLML, HLMR and LOML were developed at wavelength of 254 nm, using a mobile phase of 20% methanol, flow rate of 1 mL/min and pressure of 15 MPa. The chemical profiling of HLML, HLMR and LOML shown below indicates the metabolites present at the wavelength used and will serve as a guide in identifying the plants.

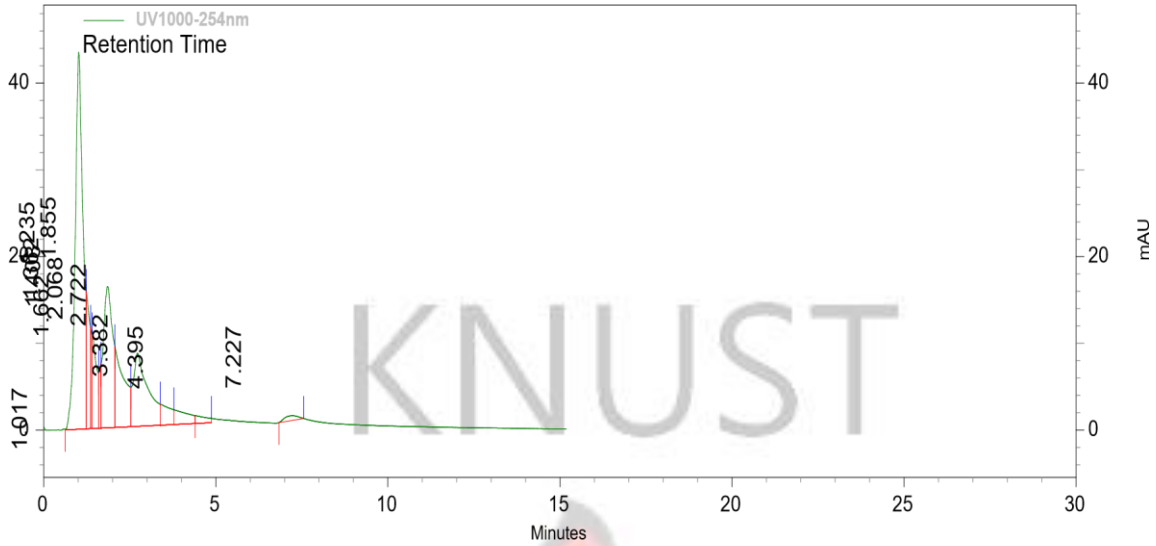

Figure 3.3.1: HPLC profile of HLML at 254 nm.

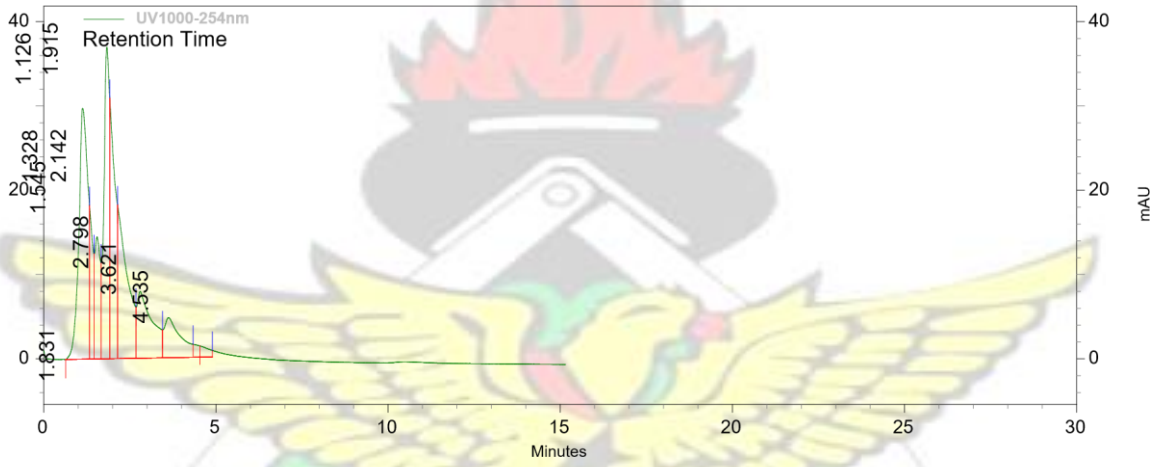

Figure 3.3.2: HPLC profile of HLMR at 254 nm.

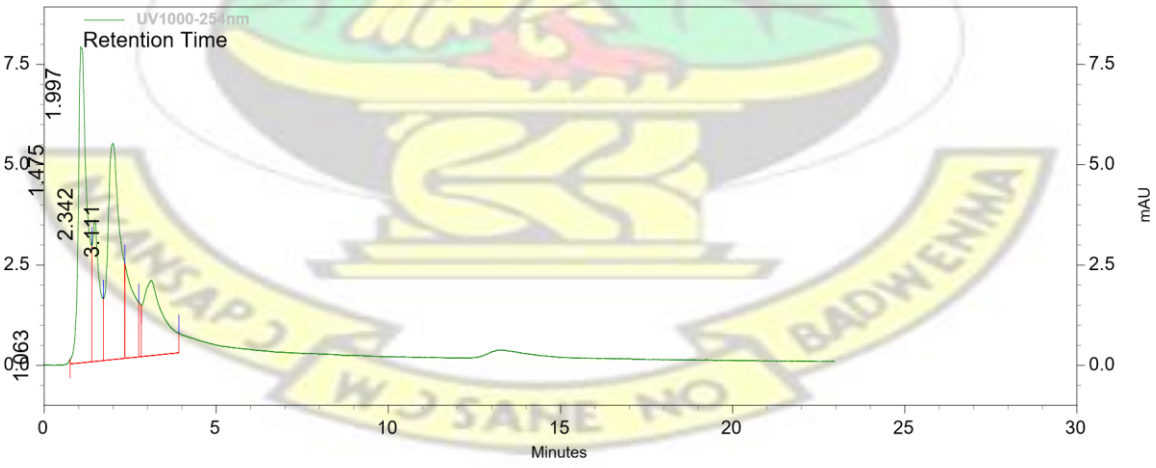

Figure 3.3.3: HPLC profile of LOML at 254 nm.

3.4 Antimicrobial activity of extracts

The broth dilution method is reproducible and does not rely on the ability of the test antimicrobial agent to diffuse through culture media making it suitable method for determination of MIC of extracts (Cos *et al.,* 2006). Hence the broth dilution method was employed to determine the antimicrobial activity and MIC of HLML, HLMR and LOML.

The MICs of HLML, HLMR and LOML against typed and clinical strains of microorganisms, consisting of Gram-positive bacteria (*B. subtilis, S. aureus, E. feacalis, S. pyogenes*), Gram negative bacteria (*E. coli, S. typhi, K. pneumonia, P. aeruginosa*) and fungus (*C. albicans*) were 50 and100 mg/mL. Whereas that of ciprofloxacin was 2 to 4 µg/mL against test bacteria and ketoconazole was 10 µg/mL against test fungi (Table 3.4.1).

Table 3.4.1 MIC of HLML, HLMR and LOML against test organisms

Test microorganisms	MIC of extracts (mg/mL)			MIC of reference antibiotics (µg/mL)	
	HLML	HLMR	LOML	Cipro	Keto
E. coli	50.0	50.0	100.0	2.0	nd
B. subtilis	50.0	50.0	50.0	2.0	nd
S. typhi	50.0	50.0	50.0	4.0	nd
K. pneumoniae	50.0	50.0	50.0	4.0	nd
S. aureus	50.0	50.0	100.0	4.0	nd
E. feacalis	50.0	100.0	100.0	4.0	nd
S. pyogenes	50.0	50.0	100.0	4.0	nd
P. aeruginosa	50.0	50.0	50.0	4.0	nd
C. albicans	50.0	100.0	100.0	nd	10.0

nd = not determined, Cipro = Ciprofloxacin, Keto = ketoconazole. Typed strains (*E. coli*, *B. subtilis*, *S. typhi*, *S. aureus, E. feacalis, P. aeruginosa*), clinical strains (*K. pneumonia*, *S. pyogenes*, *C. albicans*). HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract.

3.5 Antibiotic modifying activity by HLML and LOML

HLML and LOML ability to modify the activity of some selected antibiotics were evaluated using the broth dilution method with few modifications as described by Adu *et al*. (2014). The MICs of the antibiotics (amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin) were first determined, after which the MICs of the antibiotics were determined again in the presence of sub-inhibitory concentration (5 mg/mL) of HLML and LOML. The activity of amoxicillin against *E. coli*, *B. subtilis*, *S. typhi*, *S. aureus* and *P. aeruginosa* were potentiated in the presence of the subinhibitory concentration of HLML. The sub-inhibitory concentration of HLML again enhanced the activity of ampicillin against *E. coli* and *S. typhi*, as well as tetracycline

against *K. pneumonia*. However, both HLML and LOML sub-inhibitory concentrations reduced the activities of erythromycin and ciprofloxacin against all test organisms (Table 3.5.1).

Table 3.5.1: Effect of sub-inhibitory concentration (5mg/mL) of HLML and LOML on some antibiotics

Antibiotics only/ antibiotics + HLML/LOML		Test microorganisms							
		EC	*BS*	*ST*	*KP*	*SA*	*EF*	*SP*	*PA*
		Number of folds increase in activity							
Amoxy	HLML	8	4	2	1	8	1	>0.25	2
	LOML	2	1	0.5	0.5	2	1	>0.25	1
Ampi	HLML	2	1	4	1	1	1	>0.0625	1
	LOML	1	0.25	0.25	0.5	1	1	>0.0625	>0.0625
	HLML	0.5	1	1	4	1	1	1	2

Tetra	LOML	0.125	0.125	0.25	0.5	0.25	0.25	0.25	0.5
Erythro	HLML	0.0625	0.25	0.0625	0.125	0.0625	0.125	0.125	0.25
	LOML	0.0625	0.5	0.125	0.125	0.125	0.25	0.0625	0.125
Cipro	HLML	0.125	0.125	0.25	0.5	0.5	0.5	0.5	0.25
	LOML	0.125	0.0625	0.125	0.125	0.125	0.25	0.25	0.125

EC: E. coli, BS: B. subtilis, ST: S. typhi, KP: K. pneumonia, SA: S. aureus, EF: E. feacalis, SP: S. pyogenes, PA: P. aeruginosa. Amoxy-amoxicillin, Ampi-ampicillin, Tetra-tetracycline, Erythro-erythromycin, Cipro-ciprofloxacin, HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract.

3.6 Antioxidant activity of extracts

3.6.1 DPPH free radical scavenging activity of HLML, HLMR and LOML

HLML, HLMR and LOML ability to scavenge DPPH free radicals was studied using the method described by Prior *et al.* (2003). The method is very simple and rapid and is based on transfer of electrons or hydrogen atom by an antioxidant agent to reduce DPPH (Huang *et al*., 2005; Badarinath *et al.,* 2010). The reduced DPPH is observed as colour change from violet to yellow, which was measured spectrophotometrically at 517 nm.

HLML, LOML, HLMR and vitamin E (α-tocopherol) showed antioxidant activity at the test concentrations (0.0156 to 1 mg/mL). The IC_{50} values obtained for vitamin E, HLML, HLMR and LOML were 18.9 ± 1.3, 102.5 ± 1.5, 233.5 ± 0.5 and 130.8 ± 0.9 µg/mL, respectively (Figure 3.6.1 .2).

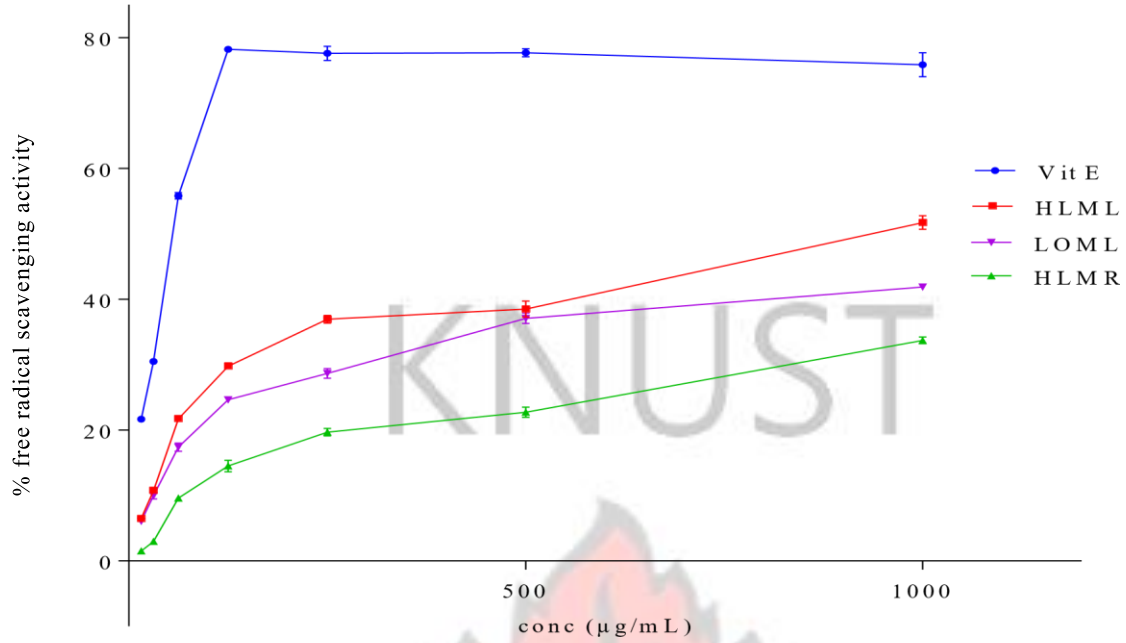

Figure 3.6.1.1: DPPH free radical scavenging activity of HLML, LOML, HLMR and standard drug (Vitamin E). conc: concentration, Vit E: Vitamin E, HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract. Values are mean ± SEM.

Table 3.6.1.1: IC_{50} of extracts and vitamin E (α-tocopherol)

Extract/ reference compound	HLML	LOML	HLMR	vitamin E
IC_{50} (µg/mL)	102.5 ± 1.477	130.8 ± 0.8511	233.5 ± 0.4933	18.90 ± 1.312

HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract. Values are mean ± SEM.

3.6.2 Total phenol content of HLML, HLMR and LOML

Folin-Ciocalteu reagent was used to assess the total phenolic content of HLML, HLMR and LOML, as described by Škerget *et al*. (2005). Phenolic compounds have the ability to reduce Folin-Ciocalteu reagent in a basic environment or medium to form a blue complex, and this can be quantified spectrophotometrically at 760 nm (Socha *et al*.,

2009). The total phenol content was calculated as milligram tannic acid equivalence (TAE) per gram of the extract using the tannic acid calibration or standard curve.

HLML, HLMR and LOML at the test concentrations (0.5 to 10 mg/mL) showed an increased phenol content with increasing concentration. HLML ranged from 10 to198 mg TAE/g of the extract, HLMR was from 27 to 175 mg TAE/g of the extract and LOML stretched from 7 to 122 mg TAE/g of the extract (Figures 3.6.2.1, 3.6.2.2 and 3.6.2.3). The total phenol content in each of the extracts (HLML, HLMR and LOML) was calculated as mean ± SEM as indicated in table 3.6.2.1, with HLML having the highest total phenol content of 103.0 ± 1.335 mg/g.

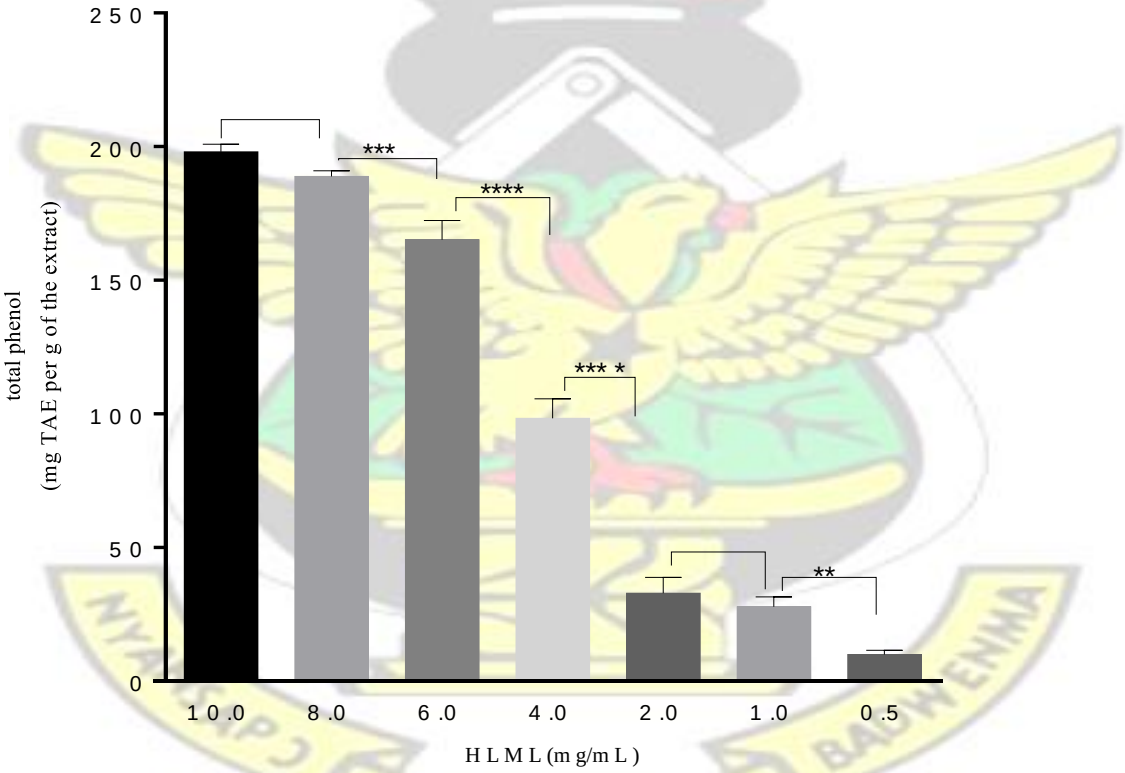

Figure 3.6.2.1: Total phenol content in different concentrations of HLML expressed as tannic acid equivalent. HLML: *Hilleria latifolia* leaf methanol extract. Values are means ± SEM ****p<0.0001; ***p< 0.001; **p< 0.01 (Tukey's *post hoc* test).

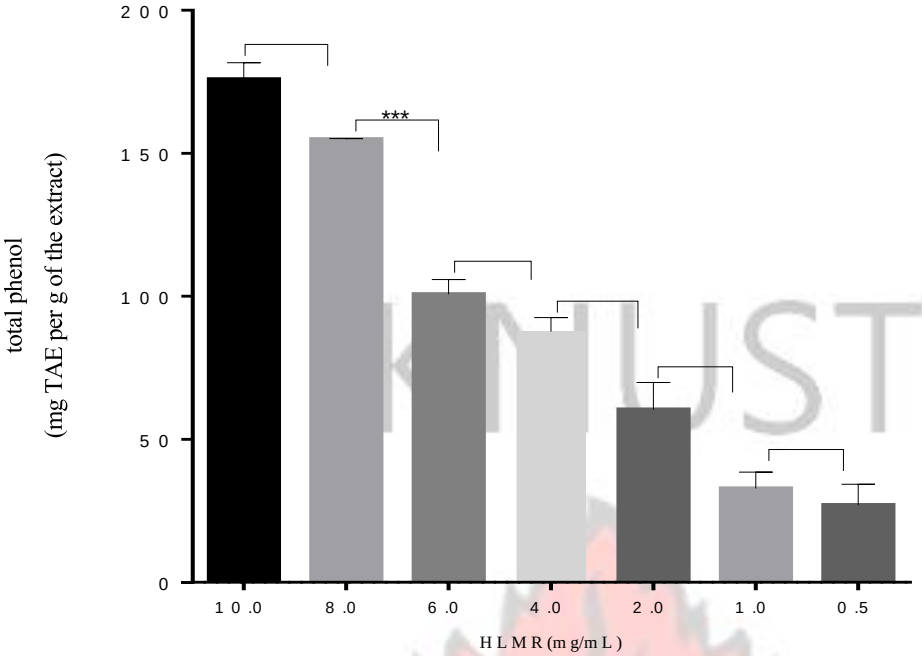

Figure 3.6.2.2: Total phenol content in different concentrations of HLMR expressed as tannic acid equivalent. HLMR: *Hilleria latifolia* root methanol extract. Values are means ± SEM. ****p*< 0.001 (Tukey's *post hoc* test).

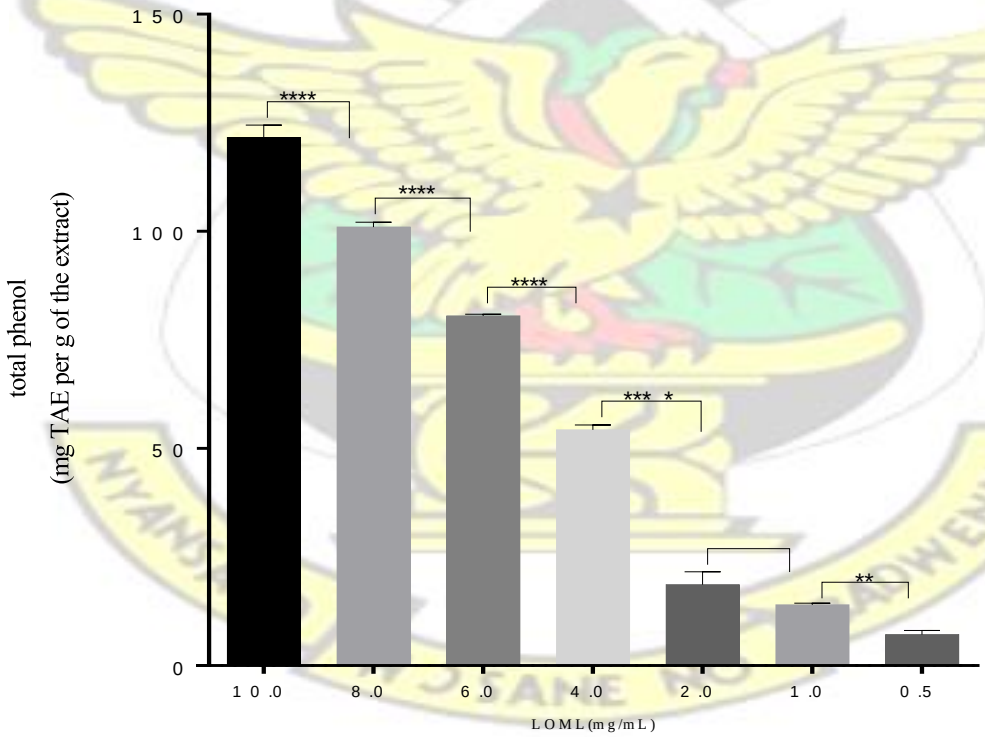

Figure 3.6.2.3: Total phenol content in different concentrations of LOML expressed as tannic acid equivalent. LOML: *Laportea ovalifolia* leaf methanol extract. Values are means ± SEM. *****p*<0.0001; ***p*< 0.01 (Tukey's *post hoc* test).

Table 3.6.2.1 Total phenol content of HLML, HLMR and LOML

Extracts	HLML	LOML	HLMR
Total phenol content (mg TAE/g extract)	103.0 ± 1.335	56.75 ± 0.3220	91.32 ± 4.258

TAE: Tannic acid equivalent, HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract. Values are mean ± SEM.

3.6.3 Total antioxidant capacity of HLML, HLMR and LOML

The phosphomolybdenum antioxidant assay as described by Prieto *et al*. (1999) and Amponsah *et al*. (2014) was employed to determine the total antioxidant capacity of HLML, HLMR and LOML, using α-tocopherol as standard drug. In an acidic condition, an antioxidant compound is able to reduce Mo^{+6} to Mo^{+5} with subsequent formation of a green complex which can be quantified by measuring absorbance at 695nm. Total antioxidant capacity of HLML, HLMR and LOML was expressed as αtocopherol equivalence (α-TE) from the α-tocopherol calibration curve.

The total antioxidant capacity of HLML was 410.4 ± 4.732 mg α-TE/g extract, HLMR was 408.0 ± 18.70 mg α-TE/g and that of LOML was calculated to be 337.6 ± 6.961 mg α-TE/g extract (Table 3.6.3.1). Values are presented as mean ± standard error mean. At the test concentrations (0.5 to 10 mg/mL) of HLML, HLMR and LOML, it was observed that, antioxidant capacity decreased as the concentrations reduced, (Figures 3.6.3.1, 3.6.3.2 and 3.6.3.3).

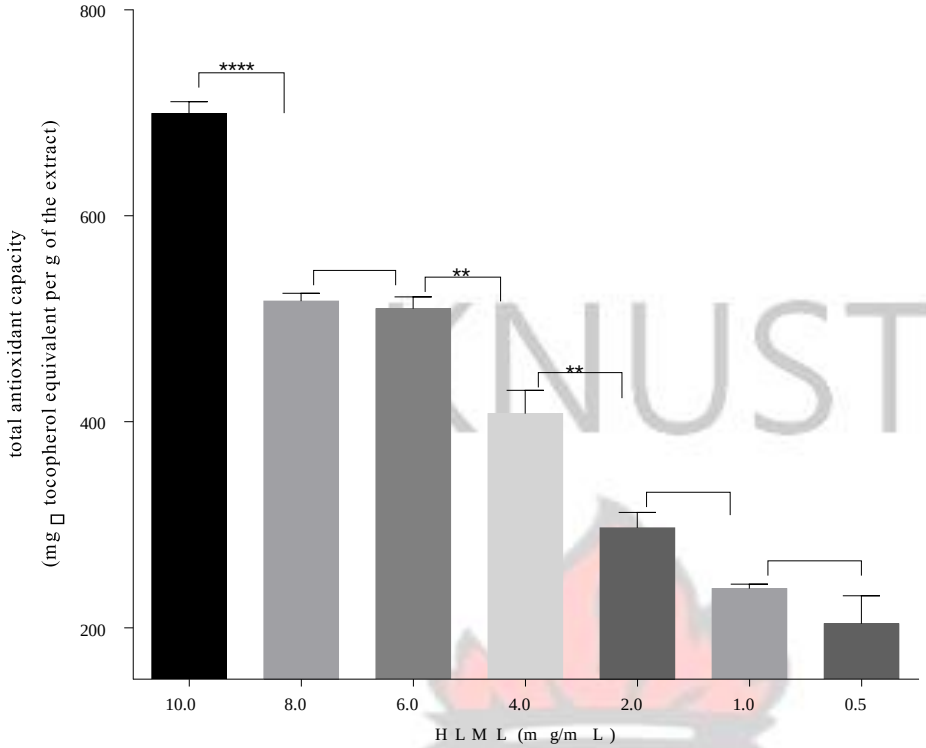

Figure 3.6.3.1: Total antioxidant capacity in different concentrations of HLML expressed as α-tocopherol equivalent. HLML: *Hilleria latifolia* leaf methanol extract. Values are means ± SEM. ****p<0.0001; **p< 0.01 (Tukey's *post hoc* test).

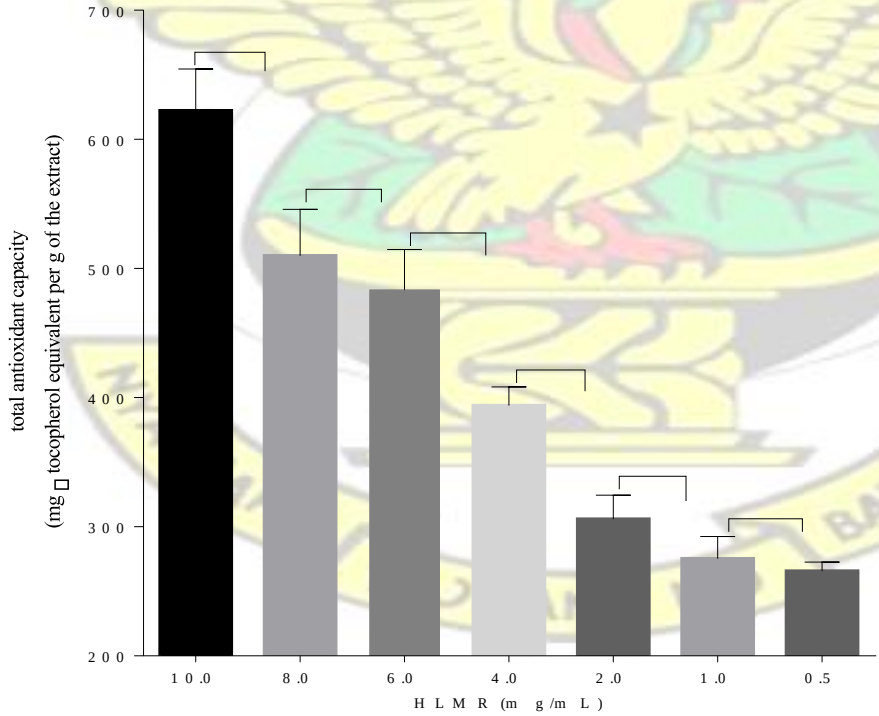

Figure 3.6.3.2: Total antioxidant capacity in different concentrations of HLMR expressed as α-tocopherol equivalent. HLMR: *Hilleria latifolia* root methanol extract. Values are means ± SEM. (Tukey's *post hoc* test).

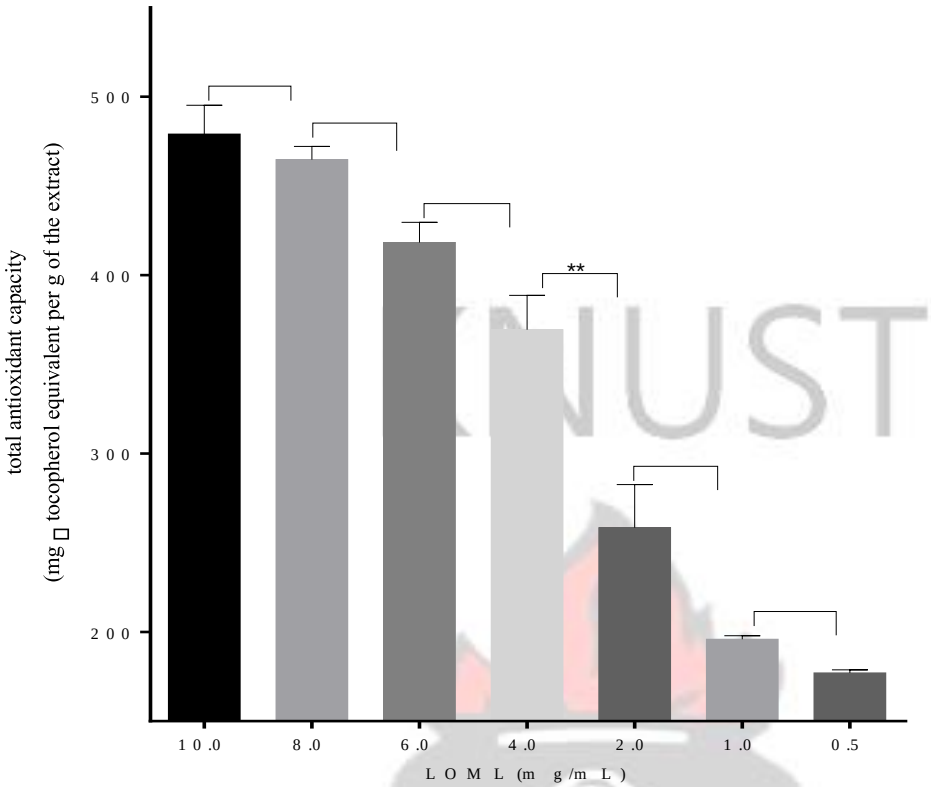

Figure 3.6.3.3: Total antioxidant capacity in different concentrations of LOML expressed as α-tocopherol equivalent. LOML: *Laportea ovalifolia* leaf methanol extract. Values are means ± SEM. $^{**}p < 0.01$ (Tukey's *post hoc* test).

Table 3.6.3.1: Total antioxidant capacity of HLML, HLMR and LOML

Extracts	HLML	LOML	HLMR
Total antioxidant capacity (mg α-TE/g extract)	410.4 ± 4.732	337.6 ± 6.961	408.0 ± 18.70

α-TE: α-tocopherol equivalent, HLML: *Hilleria latifolia* leaf methanol extract, LOML: *Laportea ovalifolia* leaf methanol extract, HLMR: *Hilleria latifolia* root methanol extract. Values are mean ± SEM.

3.7 Acute anti-inflammatory activity of HLML, HLMR and LOML

The preventive and curative effects of HLML, HLMR and LOML were examined using the carrageenan-induced foot oedema model in rats (Winter *et al.*, 1962). This model is acceptable and most widely used animal model of acute inflammation. Carrageenan has the ability to induce an intense and reproducible inflammation by causing the release of more than one inflammatory mediator which is very useful in assessing an agent's anti-

inflammatory activity (Winter *et al.,* 1962; Kaur *et al.,* 2004). Rats were grouped randomly with each group consisting of four (4) animals.

The groups were treated as follows 30, 100 and 300 mg/kg of HLML, HLMR and LOML, 100 mg/kg of aspirin (reference drug) and control group given 0.5 mL sterile distilled water (vehicle) before (preventive) or after (curative) induction of oedema.

HLML (30, 100, 300 mg/Kg) when administered before (preventive) the induction of the carrageenan paw oedema caused the mean maximal swelling attained at 2 h to be significantly ($p< 0.01$) reduced to 41.81 ± 3.14%, 51.21 ± 5.79% and 38.13 ± 3.21%, respectively from the inflamed control response of 61.50 ± 1.44% (Figure 3.7.1A). The total paw swellings induced over the 6 h (measured as the area under the time course curve, AUC) were also significantly ($p<0.001$) suppressed to 64.12 ± 4.88%, and 55.70 ± 6.14% of the inflamed control response for 30 and 300 mg/Kg (Figure 3.7.1B). Also, HLMR (30, 100 and 300 mg/Kg) showed a significant ($p<0.001$) reduction of the oedema with the mean maximal swelling attained at 2 h reduced to 54.04 ± 1.40%, 48.33 ± 0.54% and 41.30 ± 2.36% respectively, from the inflamed control response of 61.50 ± 1.44% (Figure 3.7.2A). The total paw swellings induced over the 6 h were also significantly ($p<0.001$) suppressed to 88.51 ± 4.30%, 73.38 ± 2.99% and 61.78 ± 3.64% respectively (Figure 3.7.2B). Similarly, LOML (30, 100 and 300 mg/Kg) showed a significant ($p<0.001$) reduction of the oedema with the mean maximal swelling attained at 2 h reduced respectively to 55.38 ± 0.57%, 51.15 ± 2.30% and 45.05 ± 2.81% from the inflamed control response of 61.50 ± 1.44% (Figure 3.7.3A). The total paw swellings induced over the 6 h were also significantly ($p<0.001$) suppressed to 86.96 ± 2.14%, 76.37 ± 3.70% and 67.92 ± 3.77% respectively of the inflamed control response (Figure 3.7.3B).

When administered after the induction of the carrageenan paw oedema (curative), HLML (30, 100, 300 mg/Kg) suppressed significantly (p<0.001) the mean maximal swelling attained at 4 h respectively to 61.96 ± 7.393%, 58.66 ± 1.852% and 69.62 ± 2.729% of the inflamed control response of 105.2 ± 4.553% (Figure 3.7.4A). The total paw swellings induced over the 6 h was significantly (p<0.001) suppressed to 64.63 ± 8.39%, 59.52 ± 3.46% and 69.92 ± 5.39%, respectively of the inflamed control response (Figure 3.7.4B). Similarly, HLMR (30, 100, 300 mg/Kg) showed a significant (p<0.001) inhibition of the oedema with the mean maximal swelling at 4 h reduced respectively to 63.70 ± 5.490%, 75.22 ± 7.452% and 65.70 ± 4.943% of the inflamed control response of 105.2 ± 4.553% (Figure 3.7.5A). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 64.30 ± 4.66%, 75.05 ± 9.59% and 62.00 ± 4.90% respectively of the inflamed control response (Figure 3.7.5B). Again, LOML (30, 100, 300 mg/Kg) showed a significant

(p<0.001) inhibition of the oedema with the mean maximal swelling at 4 h reduced respectively to 45.51 ± 0.6827%, 43.91 ± 1.431% and 38.19 ± 0.9960% of the inflamed control response of 105.2 ± 4.553% (Figure 3.7.6A). The total paw swellings induced over the 6 h were also significantly (p<0.001) suppressed to 48.04 ± 1.37%, 45.38 ± 1.57% and 40.79 ± 2.40% respectively of the inflamed control response (Figure 3.7.6B).

A **B**

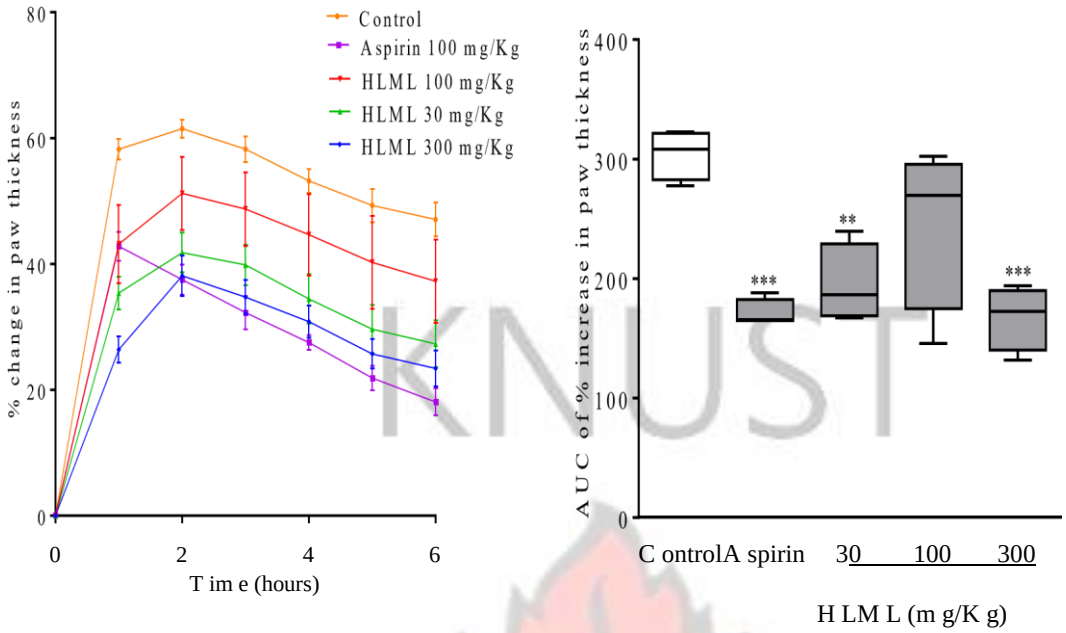

Figure 3.7.1: Prophylactic effect of HLML on carrageenan-induced oedema in rats. **A:** Timecourse curve, **B:** AUC of carrageenan induced oedema, Ctrl: Control group, Asp: Aspirin treated group, HLML: *Hilleria latifolia* leaf methanol extract. Values are means ± SEM (n=4). ***p<0.001; **p< 0.01 (Dunnett's *post hoc* test).

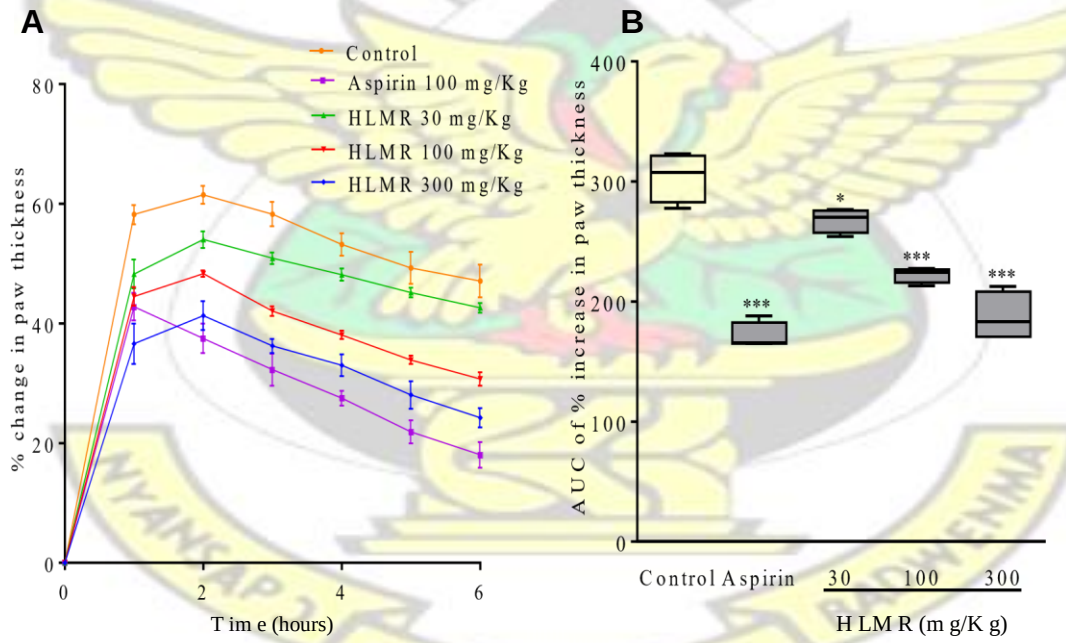

Figure 3.7.2: Prophylactic effect of HLMR on carrageenan-induced oedema in rats. **A**: Timecourse curve, **B**: AUC of carrageenan induced oedema, Ctrl: Control group, Asp: Aspirin treated group, HLMR: *Hilleria latifolia* root methanol extract. Values are means ± SEM (n=4) ***p<0.001; *p<0.05 compared to control group (Dunnett's *post hoc* test).

A **B**

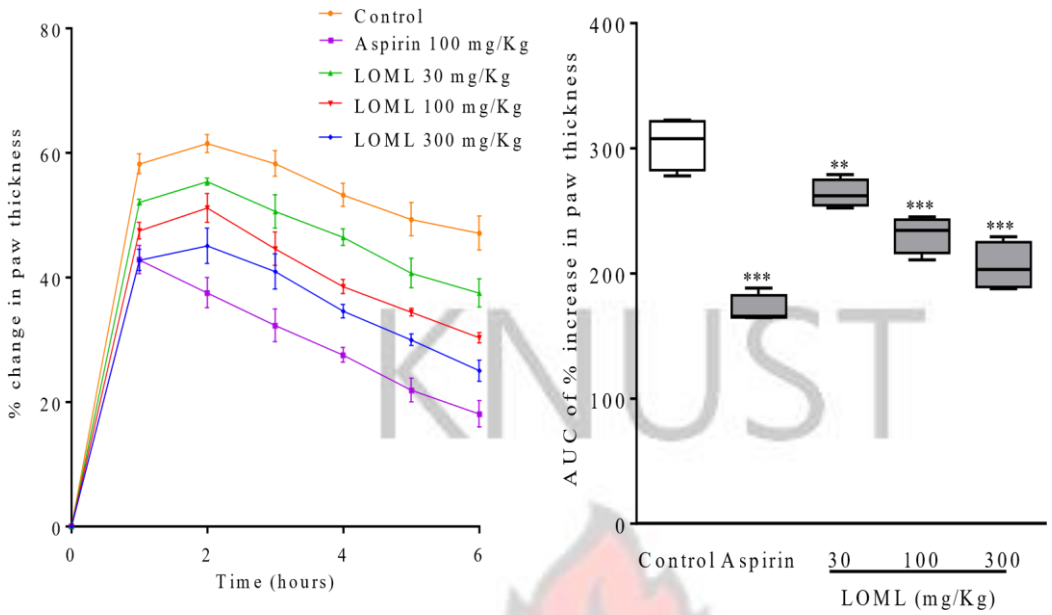

Figure 3.7.3: Prophylactic effect of LOML on carrageenan-induced oedema in rats. **A**: Timecourse curve, **B**: AUC of carrageenan induced oedema, Ctrl: Control group, Asp: Aspirin treated group, LOML: *Laportea ovalifolia* leaf methanol extract. Values are means ± SEM (n=4) ***p<0.001; **p<0.01 compared to control group (Dunnett's *post hoc* test).

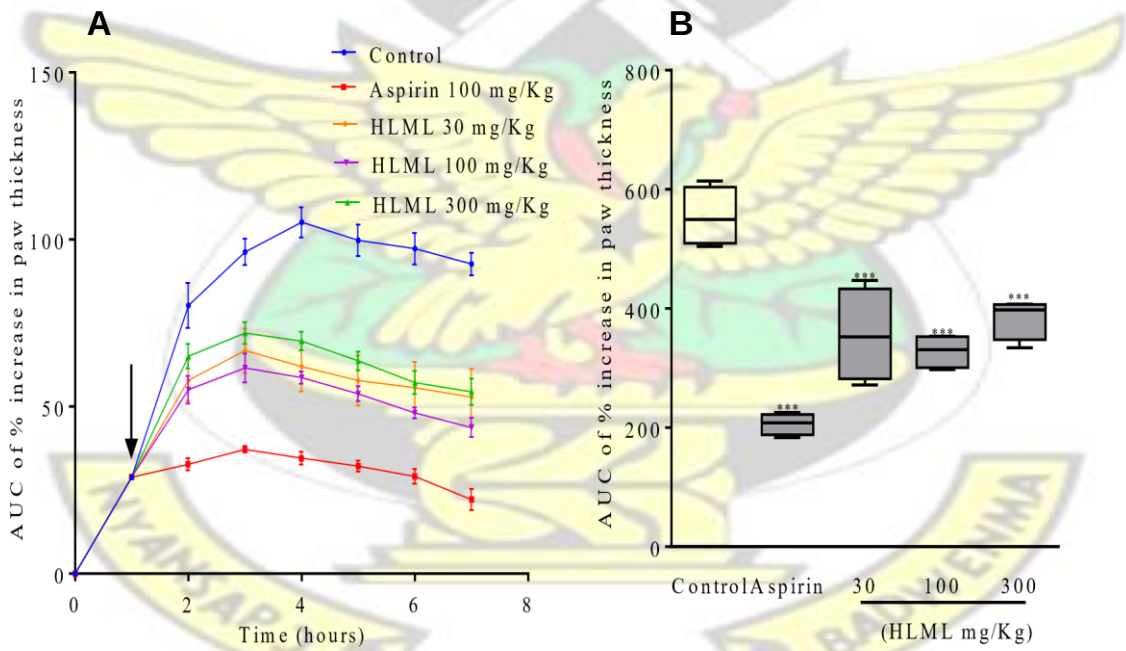

Figure 3.7.4: Therapeutic effect of HLML on carrageenan-induced oedema in rats. **A**: Timecourse curve, **B**: AUC of carrageenan induced oedema, HLML: *Hilleria latifolia* leaf methanol extract. Values are means ± SEM (n=4) ***p<0.001; compared to control group (Dunnett's *post hoc* test). Arrow indicates point of Aspirin or HLML administration.

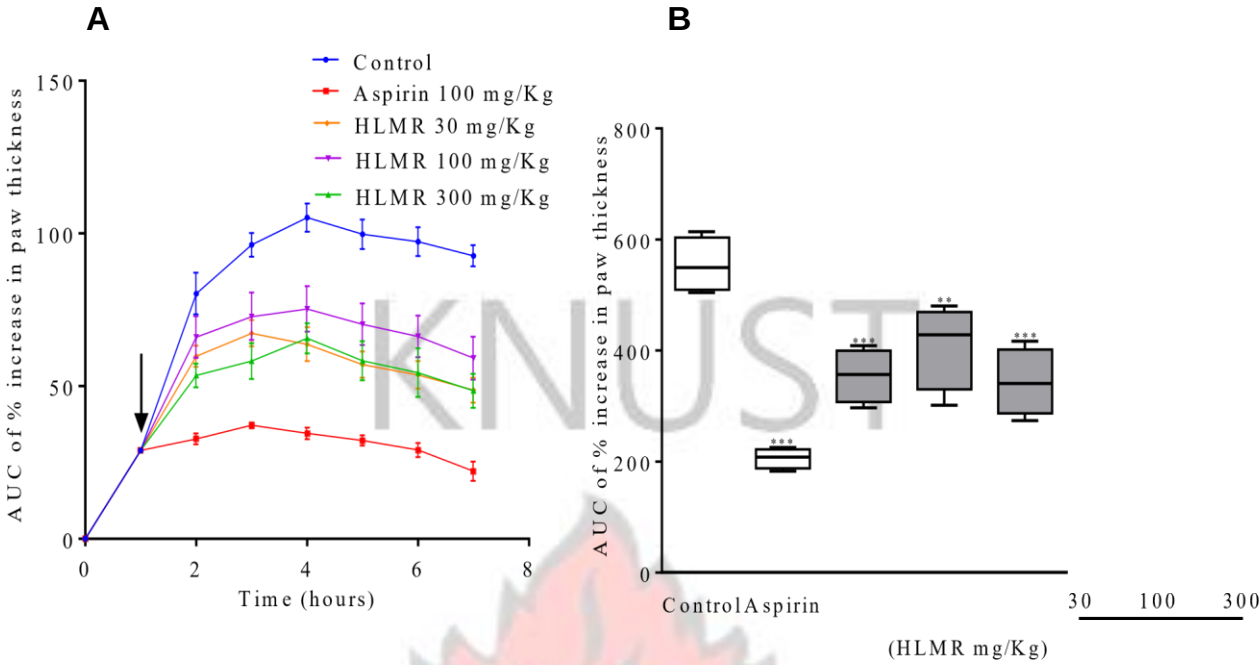

Figure 3.7.5: Therapeutic effect of HLMR on carrageenan-induced oedema in rats. **A**: Timecourse curve, **B**: AUC of carrageenan induced oedema, HLMR: *Hilleria latifolia* root methanol extract. Values are means ± SEM (n=4) ***p<0.001; **p<0.01 compared to control group (Dunnett's *post hoc* test). Arrow indicates point of Aspirin or HLMR administration.

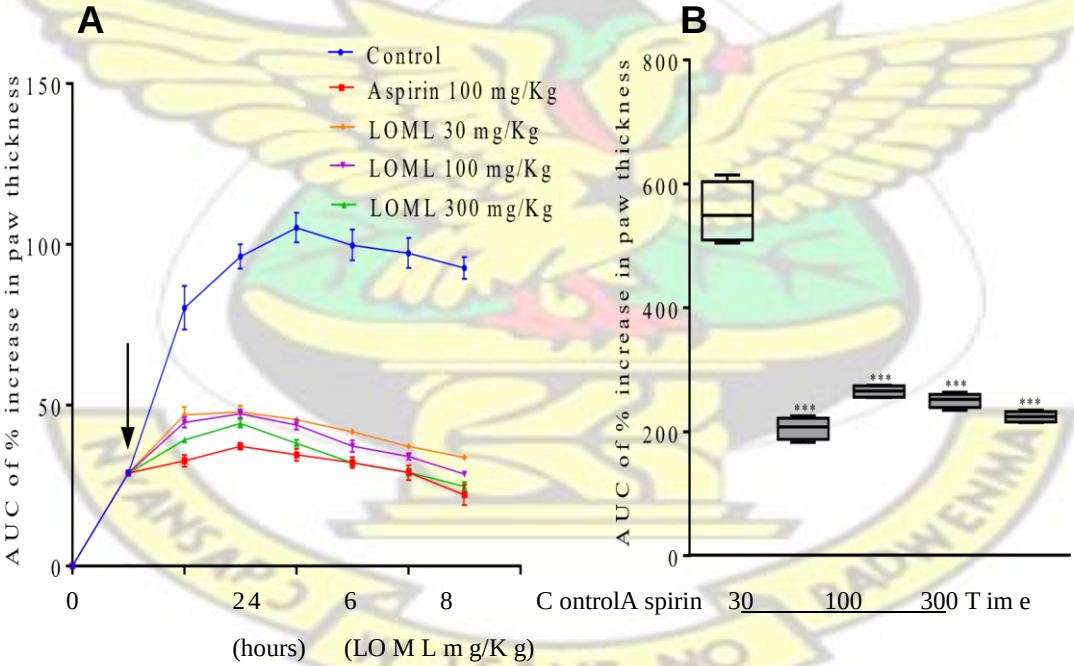

Figure 3.7.6: Therapeutic effect of LOML on carrageenan-induced oedema in rats. **A:** Timecourse curve, **B:** AUC of carrageenan induced oedema, LOML: *Laportea ovalifolia* leaf methanol extract. Values are means ± SEM (n=4) ***p<0.001 compared to control group (Dunnett's *post hoc* test). Arrow indicates point of Aspirin or LOML administration.

3.8 Wound healing activity of extracts

The excision wound model as described by Agyare *et al.* (2013c) was used to assess the influence of HLML, HLMR and LOML on wound healing. HLML, HLMR and LOML were formulated into an aqueous cream of 5 and 10% w/w each and applied on wounds daily for fifteen (15) days. Silver sulphadiazine (1% w/w) was used as reference drug, vehicle group was treated with only aqueous cream. The rate of wound closure (wound diameter) was measured every other day through the period of treatment from the day of wound creation.

HLML, HLMR and LOML at 5 and 10% significantly (p<0.001) reduced wound size from day 5 to 15 after injury (Figures 3.8.1a, 3.8.2a and 3.8.3a). Also, their respective area under the curve (AUC) revealed a significant reduction (p<0.001) in wound size when compared to the untreated group (Figures 3.8.1b, 3.8.2b and 3.8.3b).

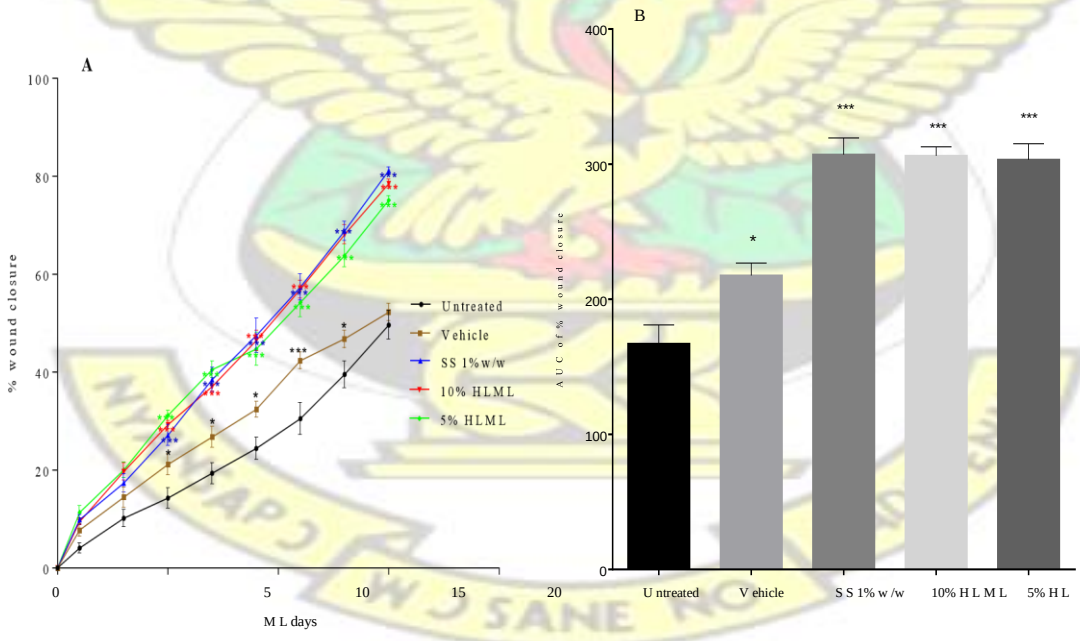

Figure 3.8.1: Influence of HLML on wound closure. **A:** time-course curve, **B:** AUC of time course curve, SS: Silver sulphadiazine, HLML: *Hilleria latifolia* leaf methanol extract. Values are mean ±SEM (n=5). ***p<0.001; **p<0.01; *p<0.05 compared to untreated group (One-way and Two-way ANOVA followed by Dunnett's and Bonferroni's *post hoc* tests, respectively).

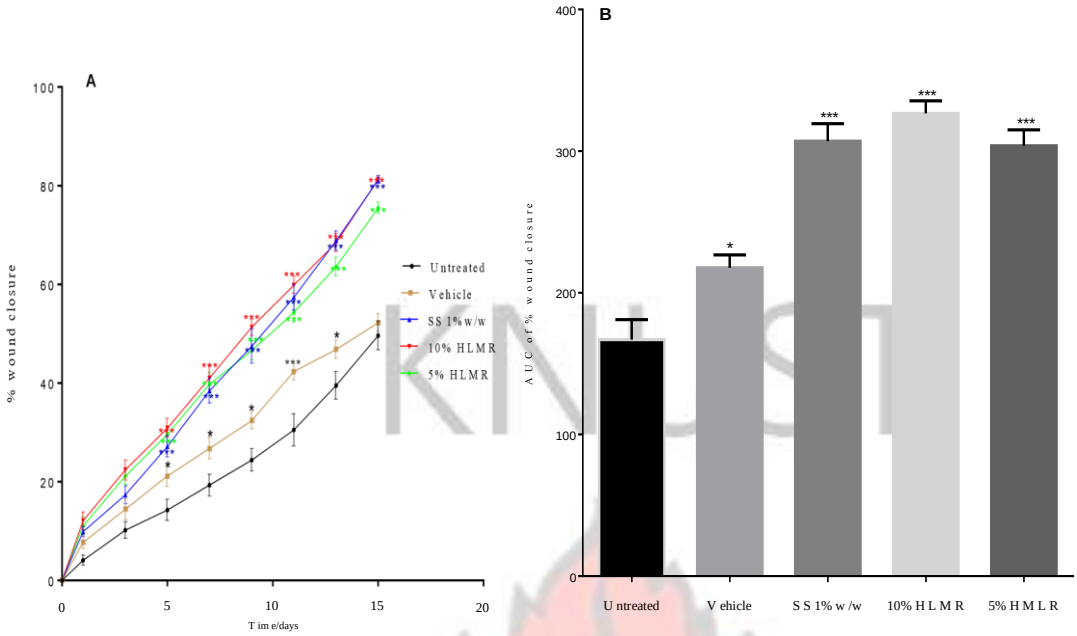

Figure 3.8.2: Influence of HLMR on wound closure. **A:** time-course curve, **B:** AUC of time course curve, SS: Silver sulphadiazine HLMR: *Hilleria latifolia* root methanol extract. Values are mean ±SEM (n=5). ***p<0.001; **p<0.01; *p<0.05 compared to untreated group (Oneway and Two-way ANOVA followed by Dunnett's and Bonferroni's *post hoc* tests, respectively).

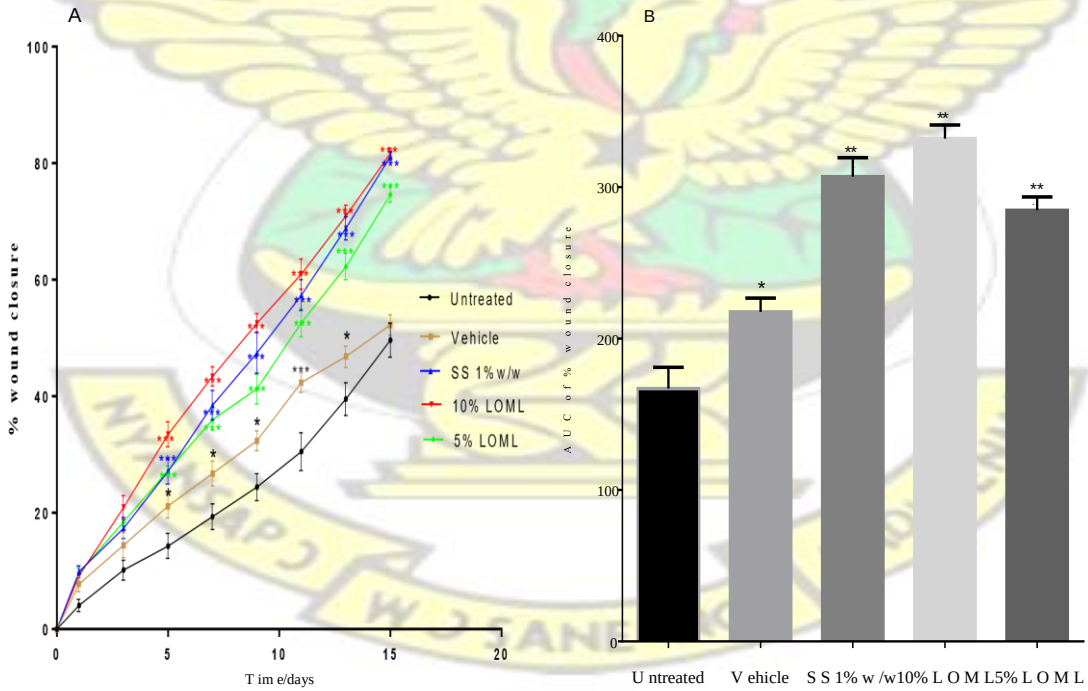

Figure 3.8.3: Influence of LOML on wound closure. **A:** time-course curve, **B:** AUC of time course curve, SS: Silver sulphadiazine, LOML: *Laportea ovalifolia* leaf methanol extract. Values are mean ± SEM (n=5). ***p<0.001; **p<0.01; *p<0.05 compared to untreated group

(One-way and Two-way ANOVA followed by Dunnett's and Bonferroni's *post hoc* tests, respectively).

3.9 Histological studies of wound tissues treated with HLML, HLMR and LOML

On the 15th day post wounding, wounds from all the groups were excised for histological examination to microscopically identify and assess morphological changes in tissues such as re-epithelisation, angiogenesis, collagenation and granular tissue formation. Heamatoxylin and eosin (H&E) staining was used for the study since the staining process is simple and also serves as a general purpose differential staining technique for differentiating between nuclear bodies and other organelles such as collagen, muscles and connective fibres in the cytoplasm (Udupa *et al.*, 1995; Chung *et al.*, 2010).

The images from the histological study revealed the following morphological characteristics:

- Untreated wound tissues revealed persistent inflammation with marked tissue necrosis and little proliferation indicating poor wound healing rate.
- Wound tissues treated with aqueous cream only (Vehicle) revealed presence of persistent inflammation and little proliferation which indicated poor wound healing rate.
- Silver sulphadiazine (1% w/w)-treated wound tissues were characterised with granulation tissue formation and angiogenesis with evidence of fibroblast proliferation and collagen synthesis indicating re-epithelialisation and wound healing.
- HLML (10%)-treated wound tissues had increased collagenation and reepithelialisation, which indicates high rate of wound healing, while HLML (5%)-treated wound tissue showed appreciable angiogenesis and fibroblastic

activity with evidence of substantial collagen deposition in wounds (Figure 3.9.1).

- HLMR (10%) treated wound tissue was marked with an increase collagenation and re-epithelisation indicative of high rate of wound healing, whereas appreciable angiogenesis and fibroblastic activity with evidence of considerable collagen deposition were observed in wound tissue treated with 5% HLMR (Figure 3.9.2) .
- Wound tissue treated with LOML (10%) showed high rate of wound healing with increased collagenation and re-epithelisation, while tissue treated with LOML (5%) indicated appreciable angiogenesis and fibroblastic activity with evidence of considerable collagen deposition in wounds (Figure 3.9.3).

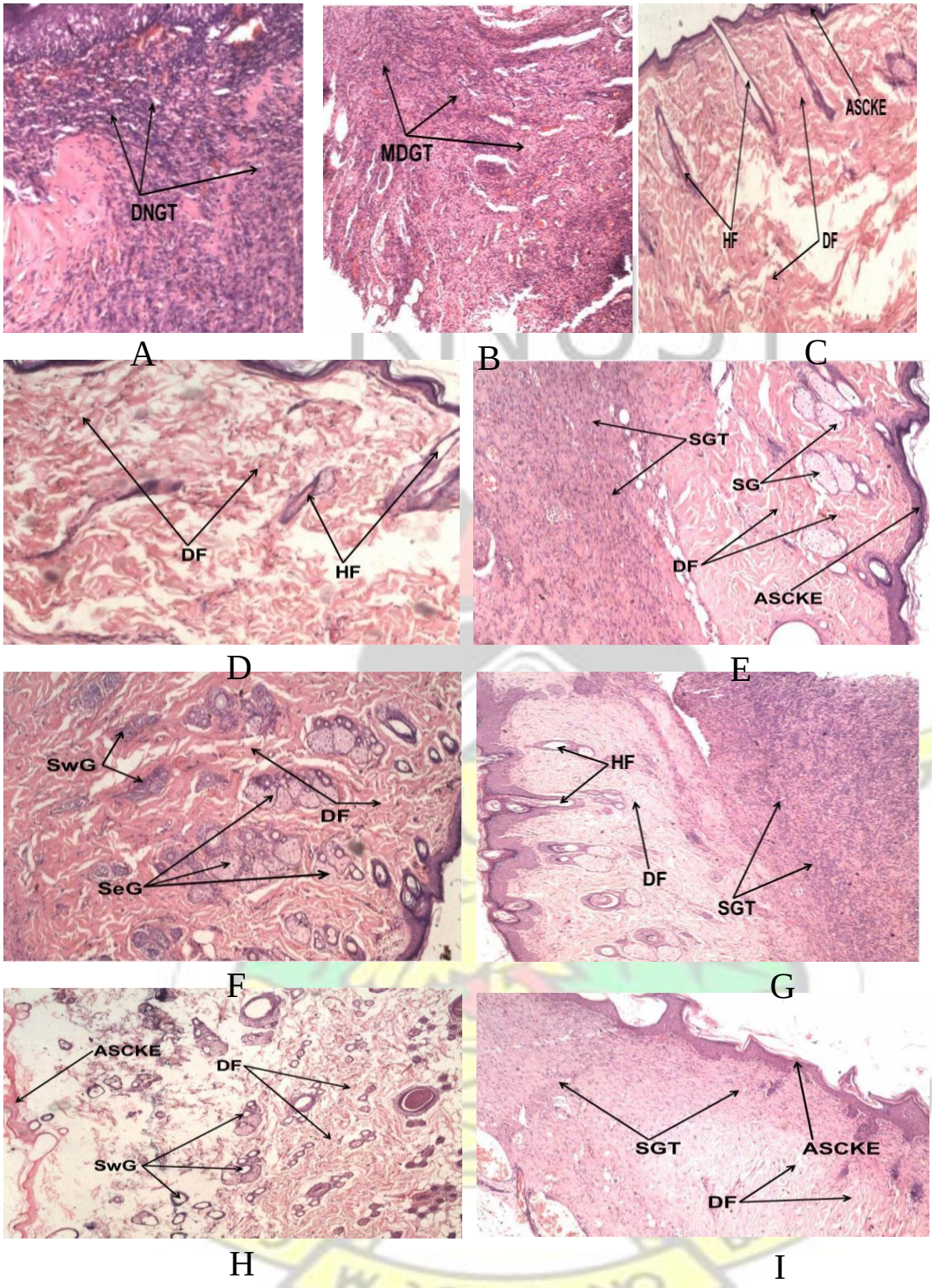

Figure 3.9.1: Histological images (x400) of wound tissues from HLML, LOML and HLMRtreated and untreated wounds. **A**: Untreated wound tissues, **B**: vehicle-treated (aqueous cream only) wound tissues, **C**: 1% w/w siver sulphadiazine-treated wound tissues, **D**: 10% w/w HLML-treated wound tissues, **E**: 5% w/w HLML-treated wound tissues, **F**: 10% w/w LOMLtreated wound tissues, **G**: 5% w/w LOML-treated wound tissues, **H**: 10% w/w HLMR-treated wound tissues, **I**: 5% w/w HLMR-treated wound tissues, **DNGT**: Diffuse necrotic granulation tissue, **MDGT**: Moderate diffuse granulation tissue, **HF**: hair follicle, **DF**: Dense

fibrous tissue, **ASCKE**: Atrophic squamous cell keratinized epithelium, **SGT**: Scanty granulation tissue, **SeG**: Sebaceous gland, **SwG**: Sweat gland.

3.10 Cell toxicity studies of extracts

3.10.1 Influence of HLML, HLMR and LOML on cell viability

The influence of HLML, HLMR and LOML on the viability of HaCaT-keratinocytes was investigated using the MTT assay as described by Agyare *et al*. (2011) and Lupu and Popescu (2013). Dehydrogenase enzymes in the mitochondria of metabolically active cells reduced yellow MTT to purple formazan, which was then quantified spectrophotometrically at 595 nm as a measure of viable cell (Niles *et al*., 2008). HaCaT-ketainocytes were treated with different concentrations (0.1, 1.0, 10.0 and 100.0 µg/mL) of HLML, HLMR and LOML to assess their influence on the viability of the cells.

Treatment with 100 µg/mL of HLML ($p<0.001$) and HLMR ($p<0.05$) significantly reduced the viability of HaCaT-keratinocytes compared to the untreated cells. However, at the lower test concentrations (0.1, 1.0 and 10.0) and LOML-treated cells, there was no significant ($p>0.05$) difference when compared to the HaCaTKeratinocyte untreated cells, whereas the cells treated with 1% foetal calf serum (positive control) significantly ($p<0.001$) increased the cells viability compared to the untreated cells (Figures 3.10.1.1 to 3.10.1.3).

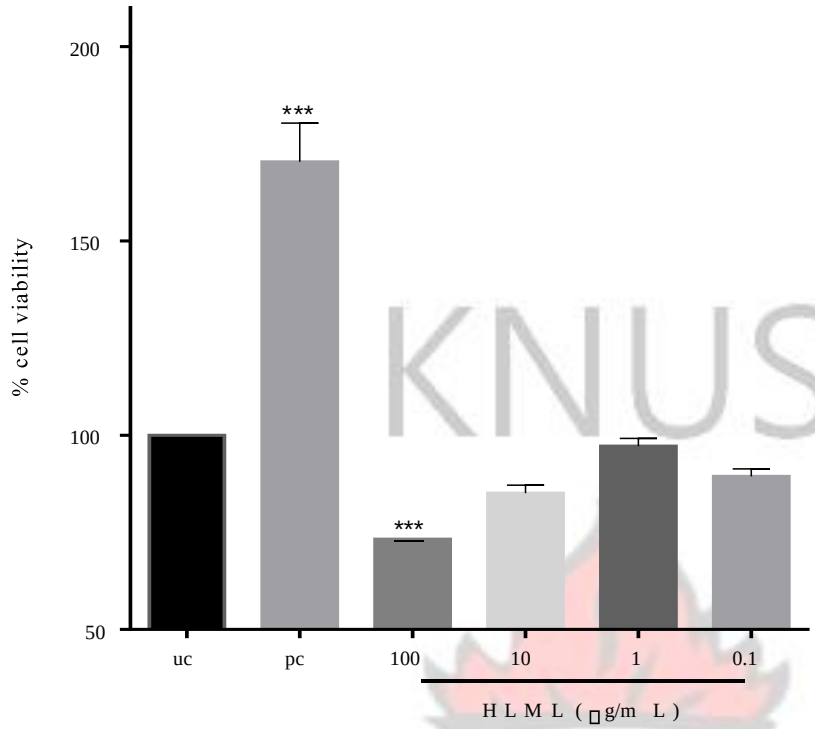

Figure 3.10.1.1: Influence of HLML on HaCaT-keratinocytes viability. uc: untreated cells; **pc**: positive control (1% foetal calf serum); HLML: *Hilleria latifolia* leaf methanol extract. Values are mean ±SEM. ***p<0.001.

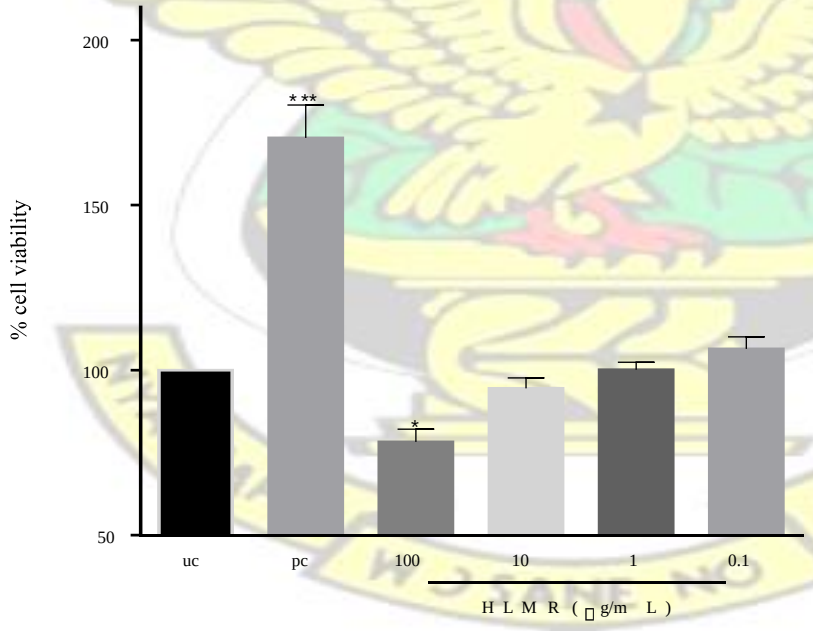

Figure 3.10.1.2: Influence of HLMR on HaCaT-keratinocytes viability. uc: untreated cells; **pc**: positive control (1% foetal calf serum); HLMR: *Hilleria latifolia* root methanol extract. Values are mean ±SEM. ***p<0.001; *p<0.05.

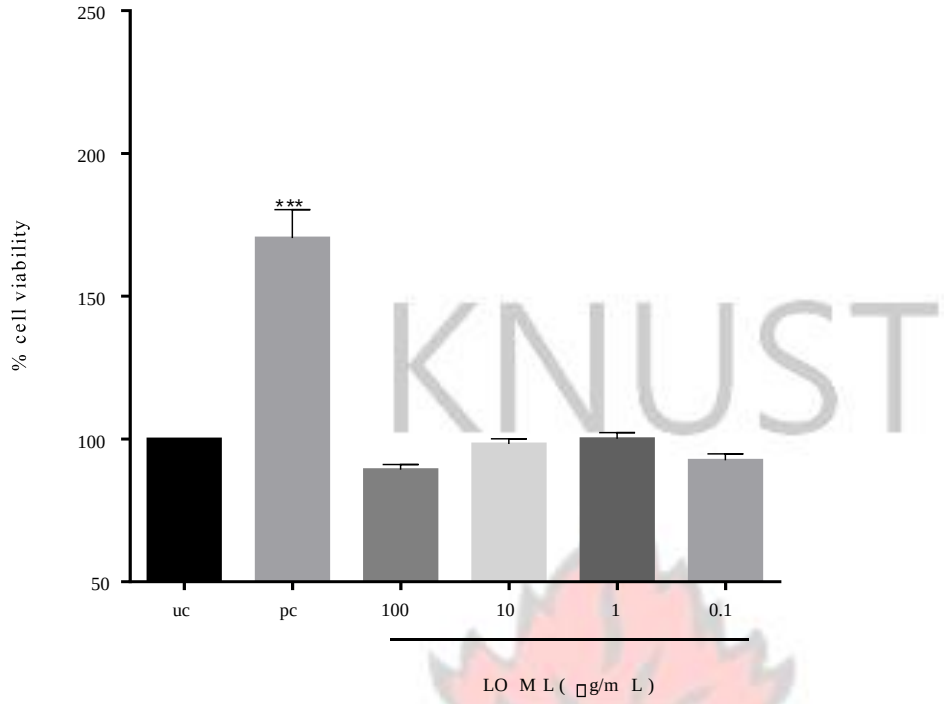

Figure 3.10.1.3: Influence of LOML on HaCaT-keratinocytes viability. uc**:** untreated cells; **pc**: positive control (1% foetal calf serum); LOML: *Laportea ovalifolia* leaf methanol extract. Values are mean ±SEM. ****p*<0.001.

3.10.2 Influence of HLML, HLMR and LOML on LDH release

The influence of HLML, HLMR and LOML on the release of LDH from HaCaTkeratinocytes was assessed to measure the integrity of the cell membrane. LDH is a stable cytoplasmic enzyme present in all cells and is only released into an extracellular medium or cell culture supernatant upon damage of the plasma membrane, indicating cell damage or necrosis. The LDH released from cells in the presence of toxic agents or compounds oxidizes lactate to pyruvate which then reacts with INT (2-[4-Iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazoliumchloride) to form red coloured formazan. The formazan was soluble in water and was quantified spectrophotometrically at a wavelength of 490 against 690 nm, as a measure of the number of lysed or damaged cells (Decker and Lohmann-Matthes, 1988; Smith *et al.,* 2011). HaCaT-keratinocytes were cultured in the presence of HLML, HLMR and

LOML (0.1, 1.0, 10.0 and 100.0 µg/mL) to assess their membrane integrity by measuring the level of LDH released. Untreated cell and cells lysed with 10% Triton X-100 in 5% FCS were used as negative and positive controls, respectively.

HLML, HLMR and LOML-treated cells at the test concentrations showed no significant (p>0.05) LDH release from HaCaT-keratinocytes compared to the untreated cells.

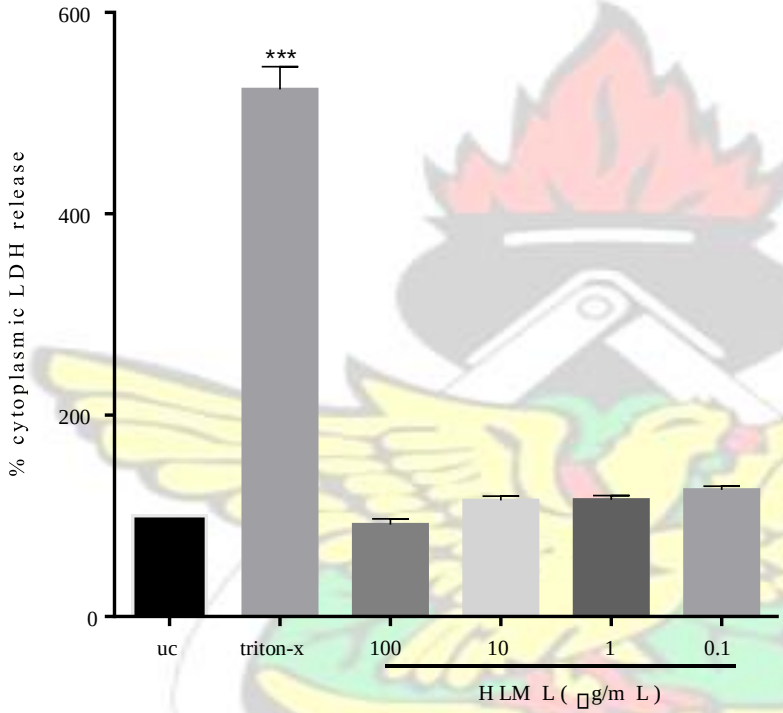

Figure 3.10.2.1: Influence of HLML on release of LDH from HaCaT-keratinocytes **uc**: untreated cells. LDH: Lactate dehydrogenase. HLML: *Hilleria latifolia* leaf methanol extract. Values are mean ±SEM. ***p<0.001.

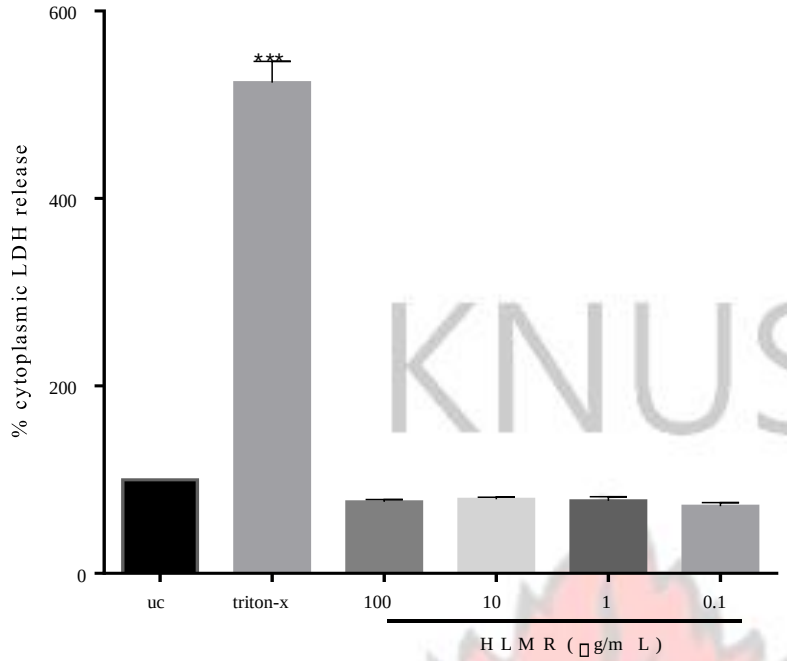

Figure 3.10.2.2: Influence of HLMR on release of LDH from HaCaT-keratinocytes **uc**: untreated cells. LDH: Lactate dehydrogenase. HLMR: *Hilleria latifolia* root methanol extract. Values are mean ±SEM. ****p*<0.001.

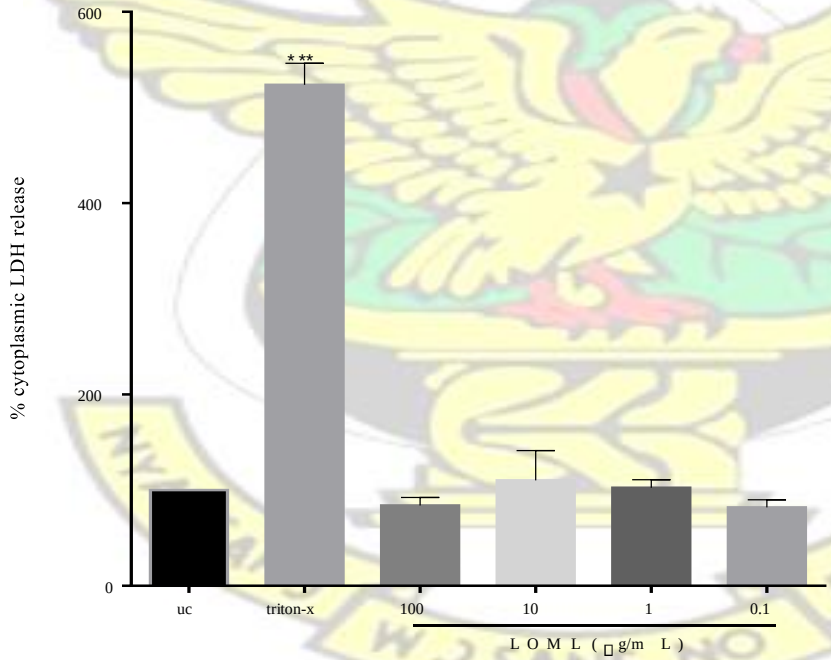

Figure 3.10.2.3: Influence of LOML on release of LDH from HaCaT-keratinocytes. **uc**: untreated cells. LDH: Lactate dehydrogenase. LOML: *Laportea ovalifolia* leaf methanol extract. Values are mean ±SEM. ****p*<0.001.

CHAPTER FOUR

4.0 DISCUSSION

Medicinal plants have been used over the years for the treatment of various ailments including infectious and metabolic (non-communicable) diseases (Ayyanar and Ignacimuthu, 2011; Calixto, 2005). Some of these plants have thoroughly been investigated and found to possess the pharmacological and biological activity for which they are traditionally used for (Agyare *et al*., 2013c). There is the need to validate or otherwise the claims of medicinal plants including *Hilleria latifolia* and *Laportea ovalifolia* used traditionally for the treatment of infections and other diseases.

H. latifolia is used traditionally for the treatment of rheumatism, boils, wounds and some skin diseases, whereas *L. ovalifolia* is used to prevent excessive menstrual bleeding and for treatment of wounds (Agyare *et al*., 2009; Mshana, 2000; Sofowora, 1996).

The preliminary phytochemical screening revealed the presence of tannins, glycosides, saponins, flavonoids, alkaloids, sterols and terpenoids in the leaves and root of *H. latifolia* similarly to what was reported by Abotsi *et al.* (2012) and Schmelzer and Gurib-Fakim, (2008). Tannins, glycosides, sterols and terpenoids were also present in *L. ovalifolia*, however, flavonoids and alkaloids were absent (Table 3.2.1). Essiett *et al.* (2011) reported the presence of phytochemicals such as tannins, glycosides, saponins, flavonoids and alkaloids. The absence of flavonoids and alkaloids in the leaves of *L. ovalifolia* may be due to the different geographical location of the plant, the season and time of collection which are all contributing factors leading to variations in the phytochemical constituents of plants of the same species (González_Martínez *et al.*, 2006; Stackpole *et al.*, 2011).

Secondary metabolites such as tannins, flavonoids, glycosides, alkaloids, terpenoids and steroids present in the plants may be responsible for their pharmacological and biological properties (Sofowora, 1993; Barbosa-Filho *et al.*, 2006; Mbagwu *et al.*, 2006; Maganha *et al.*, 2010).

Secondary metabolites in plants act individually or in synergy to bring about a wide range of pharmacological and biological activities (Gurib-Fakim, 2006; JenkeKodama *et al.*, 2008; Kubmarawa *et al*., 2008). Tannins (Buzzini *et al*., 2008; Koleckar *et al*., 2008; Fiori *et al.*, 2013), saponins (Gurib-Fakim, 2006; Murugan *et al*., 2013), alkaloids (Gomes *et al*., 2009; Singh and Kumar, 2012), flavonoids (GuribFakim, 2006; Xu and Lee, 2001), glycosides (Akdemir *et al.*, 2011) and terpenoids (Irfan *et al.*, 2014) have been reported to exhibit various pharmacological and biological activities including antimicrobial, antioxidant, anti-inflammatory, wound healing, anti-nociceptive, antiulcer, antiviral, antitumor and analgesic effects.

In addition to the phytochemical screening, HLPC profile of the 70% methanol extracts (HLML, HLMR and LOML) were developed for identification purposes. HPLC profiling is more specific and helps in easy identification and confirmation of plant on the basis of specific phytochemicals present. The profile again indicates the complex chemical composition of an extract and provides identification parameters to figure out alterations in formulated herbal products containing the extract (Tistaert *et al*., 2012).

The HPLC profiles of the extracts (Figures 3.3.1 to 3.3.3) showed that the peaks representing compounds present in the extracts appeared in the early part (early elution) of the chromatogram. This observation may be due to the polar solvent (70% methanol) used for the extraction.

The antimicrobial activity and MICs of HLML, HLMR and LOML against test organisms were determined using the broth dilution method. HLML, HLMR and LOML exhibited a broad spectrum antimicrobial activity against *E. coli B. subtilis*, *S. typhi*, *K. pneumonia*, *S. aureus*, *E. feacalis*, *S. pyogenes*, *P. aeruginosa* and *C. albicans* with MIC ranging from 50 to 100 mg/mL (Table 3.4.1). The antimicrobial activity observed may be attributed to the phytochemical constituents present in the extracts (Nweze *et al*., 2004; Edeoga *et al*., 2005).

The high MIC or low antimicrobial activity of HLML, HLMR and LOML may be as a result of low amount of the active constituents in the extracts. This observation is similar to a reported by Okwulehie and Akanwa (2013) on antimicrobial activity of *L. ovalifolia*. In this study, *L. ovalifolia* at 50 mg/ml did not inhibit the growth of the test organisms. However, Assob *et al*. (2011) reported on the antimicrobial activity of *H. latifolia* with MIC of 0.6 to 2.5 mg/mL. The MIC of 50 to 100 mg/mL for *H. latifolia* observed in this study may be as a result of different extraction procedures used and different locality of the plant materials used which may lead to different composition in terms of primary and secondary metabolites. The MIC of HLML against all the test microorganisms was 50 mg/mL, while that of HLMR was 100 mg/mL against *E. feacalis* and *C. albicans*, and 50 mg/mL against the remaining test organisms.

Fabry *et al.*, (1998) and Navarro and Delgado, (1999) reported that plant extracts with MIC ranging between 2.5 and 8 mg/mL are likely to be potential source of strong antimicrobial compounds. This suggests that HLML, HLMR and LOML may not be potential source of effective antimicrobial compounds. It is also well noted that plant extracts with low antimicrobial activity may contain some phytoconstituents that can modify the antimicrobial activity of known antimicrobials especially against resistant bacteria (Adu *et al* 2009).

Sub-inhibitory concentration of 5 mg/mL HLML and LOML modified the activity of amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin by either potentiating or nullifying their activity against the test organisms. The sub-inhibitory concentration (5 mg/mL) of HLML and LOML enhanced the activity of amoxicillin against most of the test organisms. The activity of ampicillin and tetracycline against *E. coli*, *S. typhi* and *K. pneumonia*, *P. aeruginosa* respectively were potentiated in the presence of sub-inhibitory concentration of HLML. Erythromycin and ciprofloxacin lost their activities completely against all the test organisms (Table 3.5.1).

The increased activity of these antibiotics in the presence of the sub-inhibitory concentration may be attributed to the phytochemicals of the extracts. For instance, flavonoids have been reported to have the ability to reverse the resistance of *S. aureus* to some antibiotics (Aiyegoro *et al*., 2009). Antimicrobials from plants, at subinhibitory concentrations, have been reported to be efficient in synergism with antibiotics by enhancing their antimicrobial actions (Kamatou *et al*., 2006). The phytochemicals act by reversing the resistance mechanisms of some microorganisms, thereby rendering them susceptible to antibiotics (Tenover, 2006). Plants have also been known to produce multi-drug resistance inhibitors (MDRIs) to enhance the antimicrobial activities of compounds (Stermitz *et al*., 2000).

The reduced or nullified activity of the antibiotics may be as a result of interactions between the phytochemicals in the extract and the antibiotics or the microorganisms. The phyto-constituents may react chemically with the antibiotics leading to loss of activity (Adu *et al*., 2009; Adu *et al*., 2014). It has been established that certain substances can shield microorganisms from the lethal effects of some antimicrobial agents (Keweloh *et al*., 1989). For example, some phytochemicals can bind to the surface structures of microorganisms thereby reducing their permeability to

antibiotics (Adu *et al*., 2014).

Furthermore, some of the phytocontituents may act as protein activators or coenzymes which bind to and activate enzymes responsible for resistance in an organism, making them resistant to a previously potent antibiotic (Lambert, 2002).

Antioxidant activity of HLML, HLMR and LOML was determined by assessing their DPPH free radical scavenging properties, total phenol content and total antioxidant capacity. The DPPH free radical scavenging activity of HLML, LOML and HLMR increased with increasing concentration (Figure 3.6.1.1).

The IC_{50} gives an idea on the ability of an agent to mop up free radicals indicating its potency as an antioxidant. Lower IC_{50} values indicate potent antioxidant activity (Apenteng *et al*., 2014). The IC_{50} values of the extracts (Table 3.6.1.1) indicate that HLML (102.5 ± 1.5 µg/mL) exhibited the highest antioxidant activity, followed by LOML (130.8 ± 0.9 µg/mL) and HLMR (233.5 ± 0.5 µg/mL) having the least antioxidant property.

The antioxidant activity observed in HLML, LOML and HLMR may be due to their phenolic and/or non-phenolic contents (Wu and Ng, 2008). Moreover, the highest antioxidant activity of HLML as reported by Abosti *et al*. (2012) may be due to the presence of flavonoids. Even though HLMR exhibited an antioxidant activity, it was not as potent as the HLML, since the IC_{50} of HLMR was higher than that of HLML.

This may be due to absence of phenolic compounds like flavonoids in HLMR (Jain *et al.*, 2008; Belguidoum *et al.*, 2015).

For this reason, the total phenol content of HLML, LOML and HLMR was determined. From the result, HLML had the highest total phenol content of 103.0 ± 1.335 mg/g,

which may also contribute to its free radical scavenging activity. Redox properties of phenolic compounds play an important role in neutralizing free radicals, quenching singlet and triplet oxygen species, or decomposing peroxides (Rice-Evans *et al*., 1996; Zheng and Wang, 2001). HLMR had higher total phenol content (91.32 ± 4.258 mg/g) as compared to LOML of (56.75 ± 0.3220 mg/g). This indicates that the higher free radical scavenging activity of LOML as compared to HLMR may be due to other non-phenolic compounds present (Conforti *et al*., 2008).

The total antioxidant capacity of HLML, LOML and HLMR (410.4 ± 4.732, 337.6 ± 6.961 and 408.0 ± 18.70 mg/g, respectively) correlated with their respective total phenol content. This indicates that the higher the total phenol content the greater the total antioxidant capacity of the extracts. This confirms the report that flavonoids and other phenolic compounds significantly cause the reduction of Mo^{+6} to Mo^{+5} (Khan *et al.*, 2012).

The acute anti-inflammatory activity of HLML, LOML and HLMR was determined using the carrageenan-induced oedema in rat foot model (Winter *et al.*, 1962). Carrageenan-induced oedema is a vascular response with exudation of inflammatory cells and fluids at the site of injury, caused by dilation of arterioles and eventual increase in permeability of post capillary venules (Vinegar *et al*., 1987).

The molecular response to carrageenan-induced oedema is bi-phasic involving the release of diverse inflammatory mediators, characterised by marked oedema formation. Inflammatory mediators such as histamine, serotonin and bradykinin are released during the first phase (1 to 2 h), and sustained by the release of prostaglandins and nitric oxide in the second phase (Thomazzi *et al*., 2010; Abosti *et al*., 2012).

The events involved in the vascular response to carrageenan-induced oedema are similar to the early exudative stage of inflammation (Winter *et al*., 1962; Ozaki, 1990) and hence the use of anti-inflammatory agent to inhibit this acute phase of inflammation mitigates the inflammatory process. However, it must be noted that, anti-inflammatory activity of a drug or an agent administered prophylactically does not certainly imply its ability to work therapeutically. For instance, cyclosporine prevented the inception of collagen-induced inflammation in rats but could not treat it after the inception of inflammation (Kaibara *et al*., 1983).

HLML, LOML and HLMR significantly ($p<0.001$) at 300 mg/kg reduced the induced oedema during both prophylaxis and therapeutic treatments. This indicates the presence of compounds that can reduce the inflammatory responses. Anti-inflammatory activity of ethanol leaf extract of *H. latifolia* has also been reported by Abosti *et al*. (2012), using the carrageenan induced oedema in 7-day old chicks model. In addition to the leaves of *H. latifolia*, the anti-inflammatory activity of the root was also determined using the carrageenan induced oedema in rats. The root extract (HLMR) also exhibited anti-inflammatory activity by significantly ($p<0.001$) reducing induced oedema at 300 and 100 mg/kg in both prophylactic and therapeutic experiments.

Even though the mechanisms of action of HLML, LOML and HLMR are not known, they could be acting by inhibition and/or interference in the action of inflammatory mediators (such as histamine, serotonin, bradykinin, prostaglandins and other cyclooxygenase products) involved in the carrageenan induced oedema (Abotsi *et al*., 2012; Obiri *et al*., 2013).

Also phytochemicals such as tannins, glycosides, sterols, terpenoids, flavonoids and alkaloids present in HLML, LOML and HLMR may contribute to their anti-inflammatory activities. Some of these secondary metabolites present in plant extracts have been reported to exhibit anti-inflammatory properties (Guardia *et al*., 2001; Rotelli *et al*., 2003; Barbosa-Filho *et al*., 2006).

Furthermore, the mechanism of anti-inflammatory action of HLML, LOML and HLMR may also be due to their antioxidant properties. ROS are released from activated neutrophils and macrophages during inflammatory injury and their overproduction leads to tissue injury or damage. ROS can also cause the release of pro-inflammatory cytokines such as IL-1 and TNF-α which directly enhance inflammatory response and also stimulate the production of neutrophils and macrophages (Dinarello, 2000; Conforti *et al*., 2008). Hence, the extracts ability to neutralize the effects of ROS and free radicals can contribute to their antiinflammatory activities.

The ethnomedicinal use of *H. latifolia* and *L. ovalifolia* as wound healing agents (Agyare *et al*., 2009) was investigated using the excision wound model in rats. Wounds treated with 5 and 10% HLML, HLMR and LOML formulated aqueous creams enhanced high rate of wound contraction as indicated by centripetal movement of the edges of a full-thickness wound (Suguna *et al.*, 2002, Tang *et al.*, 2007). Rate of wound contraction in excised wounds treated with 5 and 10% w/w of HLML, HLMR and LOML significantly ($p<0.001$) increased from day five (5) up to day fifteen (15) when compared with the untreated group (Figures 3.8.1 to 3.8.3).

Again, histological investigations of the treated wounds revealed high fibroblast proliferation, angiogenesis and granulation, collagenation, tissue formation and reepithelisation in treated wound tissues compared to the untreated wound tissues.

Wound contraction is indicated by enhanced re-epithelialisation, granulation, angiogenesis, fibroblast proliferation, keratinocyte differentiation and proliferation (Tang *et al.*, 2007).

The wound healing activity of HLML, HLMR and LOML may be attributed to their antioxidant and anti-inflammatory properties. Over-production of ROS and prolonged inflammation can result in impaired wound healing (Martin and Leibovich, 2005; Shetty *et al.*, 2008). Therefore an agent ability to mop up excess ROS or free radicals can minimize the inflammatory response at wound site which can also enhance the wound healing process.

During development of human skin towards an intact barrier system, keratinocytes will undergo cellular proliferation followed by a switch to cellular differentiation to restore the breakage in the intact skin (Agyare *et al.*, 2011). HLML ($p<0.001$) and HLMR ($p<0.01$) at 100 µg/mL significantly reduced the viability of HaCaTkeratinocytes compared to the untreated cells. This may suggest that HLML and HLMR may be cytotoxic at higher concentrations. Also, HLML, HLMR and LOML, though not significant ($p>0.05$), inhibited the release of LDH from the cytoplasm of HaCaT-keratinocytes compared to the untreated cells. Low LDH release from the cytoplasm by the extracts suggests that HLML, HLMR and LOML may not be cytotoxic agents at the concentrations used. There is a need to isolate and identify the bioactive compounds in the extracts responsible for the above biological activities.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The leaf and root extracts of *H. latifolia* and the leaf extract of *L. ovalifolia* exhibited antimicrobial, antioxidant, anti-inflammatory and wound healing activities. HLML, HLMR and LOML showed a broad spectrum antimicrobial activity against test organisms at high concentrations. The sub-inhibitory concentration of the extracts also modified the activity of some antibiotics by either enhancing or reducing their antimicrobial activity. HLML, HLMR and LOML exhibited both prophylactic and therapeutic acute anti-inflammatory activities. The extracts did not show cytotoxic effect on HaCaT-keratinocytes at the concentration used.

5.2 RECOMMENDATIONS

- The mechanism of action of the extracts for anti-inflammatory activity should be investigated.
- *In vivo* toxicity studies should be conducted on the plants to establish their toxicity potentials in living tissues.
- Active compounds responsible for anti-inflammatory and wound healing properties should be isolated and characterized.

REFERENCES

Abotsi, W. K. M., Ainooson, G. K. and Woode, E. (2012). Anti-inflammatory and antioxidant effects of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt.(Phytolaccaceae). *African Journal of Traditional, Complementary and Alternative Medicines,* 9(1):138–152.

Adu, F., Gbedema, S. and Annan, K. (2009). Antimicrobial and resistance modulatory activities of *Corynanthe pachyceras*. *Pharmacognosy Research,* 1(5):280–284.

Adu, F., Gbedema, S. Y., Akanwariwiak, W. G., Annan, K. and Boamah, V. E. (2011). The effects of *Acanthospermum hispidum* extract on the antibacterial activity of amoxicillin and ciprofloxacin. *Hygeia Journal for Drugs and Medicines*, 3(1):58–63.

Adu, F., Sam, G. H., Agyare, C., Apenteng, J. A., Boamah, V. E. and NtinagyeiMintah, D. (2014). Influence of methanol fruit and leaf extracts of *Myristica fragrans* (Myristicaceae) on the activity of some antibiotics. *African Journal of Microbiology Research*, 8(19):1982–1986.

Agyare, C., Asase, A., Lechtenberg, M., Niehues, M., Deters, A. and Hensel, A. (2009). An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. *Journal of Ethnopharmacology*, 125(3):393–403.

Agyare, C., Bempah, S. B., Boakye, Y. D., Ayande, P. G., Adarkwa-Yiadom, M. and Mensah, K. B. (2013c). Evaluation of antimicrobial and wound healing potential of *Justicia flava* and *Lannea welwitschii*. *Evidence-Based Complementary and Alternative Medicine*, 2013.

Agyare, C., Dwobeng, A. S., Agyepong, N., Boakye, Y. D., Mensah, K. B., Ayande, P. G. and Adarkwa-Yiadom, M. (2013a). Antimicrobial, antioxidant, and wound healing properties of *Kigelia africana* (Lam.) Beneth. and *Strophanthus hispidus* DC. *Advances in Pharmacological Sciences*, 2013.

Agyare, C., Koffuor, G. A., Boakye, Y. D. and Mensah, K. B. (2013b). Antimicrobial and anti-inflammatory properties of *Funtumia elastica*. *Pharmaceutical biology*, 51(4):418–425.

Agyare, C., Koffuor, G. A., Boamah, V. E., Adu, F., Mensah, K. B. and Adu-Amoah, L. (2012). Antimicrobial and anti-inflammatory activities of *Pterygota macrocarpa* and *Cola gigantea* (Sterculiaceae). *Evidence-Based Complementary and Alternative Medicine*, 2012.

Agyare, C., Koffuor, G. A., Mensah, A. Y. and Agyemang, D. O. (2006a). Antimicrobial and uterine smooth muscle activities of *Albizia ferruginea* extracts. *Boletin latinoamericano y del caribe de plantas medicinales y aromaticas*, 5(2):31–35.

Agyare, C., Lechtenberg, M., Deters, A., Petereit, F. and Hensel, A. (2011). Ellagitannins from *Phyllanthus muellerianus* (Kuntze) Exell.: Geraniin and furosin stimulate cellular activity, differentiation and collagen synthesis of human skin keratinocytes and dermal fibroblasts. *Phytomedicine*, 18(7):617–624.

Agyare, C., Mensah, A. Y. and Osei-Asante, S. (2006b). Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y aromaticas*, 5(6):113–117.

Agyare, C., Owusu-Ansah, A., Ossei, P. P. S., Apenteng, J. A. and Boakye, Y. D. (2014). Wound healing and anti-infective properties of *Myrianthus arboreus* and *Alchornea cordifolia*. *Medicinal Chemistry*, 4(7):533–539.

Aiyegoro, O. A., Afolayan, A. J. and Okoh, A. I. (2009). Synergistic interaction of *Helichrysum pedunculatum* leaf extracts with antibiotics against wound infection associated bacteria. *Biological research*, 42(3):327–338.

Akdemir, Z., Kahraman, Ç., Tatlı, I. I., Akkol, E. K., Süntar, I. and Keles, H. (2011). Bioassay-guided isolation of anti-inflammatory, antinociceptive and wound healer glycosides from the flowers of *Verbascum mucronatum* Lam. *Journal of ethnopharmacology*, 136(3):436–443.

Akharaiyi, F. C. (2011). Antibacterial, phytochemical and antioxidant activities of *Datura metel*. *International Journal of PharmTech Research*, 3(1):478–483.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C. and Fasure, K. A. (2005). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC complementary and alternative medicine*, 5(1):6.

Amponsah, I. K., Annan, K., Koffuor, G. A., Adusei, J., Umerie, I. J. and Osei-wusu, S. (2014). Anti-inflammatory and antioxidant properties of the ethanolic stem bark extract of *Artocarpus altilis* (Parkinson) Fosberg (Moraceae). *Der Pharmacia Lettre*, 6(3):211–217.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48(1):5–16.

Anesini, C. and Perez, C. (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. *Journal of Ethnopharmacology*, 39(2):119–128.

Apenteng, J. A., Agyare, C., Adu, F., Ayande, P. G. and Boakye, Y. D. (2014). Evaluation of wound healing potential of different leaf extracts of *Pupalia lappacea*. *African Journal of Pharmacy and Pharmacology*, 8(41):1039–1048.

Assob, J. C. N., Kamga, H. L. F., Nsagha, D. S., Njunda, A. L., Nde, P. F., Asongalem, E. A., Njouendou, A. J., Sandjon, B. and Penlap, V. B. (2011). Antimicrobial and toxicological activities of five medicinal plant species from Cameroon Traditional Medicine. *BMC complementary and alternative medicine*, 11(1):70.

Ayoola, G. A., Coker, H. A., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C. and Atangbayila, T. O. (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research*, 7(3):1019–1024.

Ayyanar, M. and Ignacimuthu, S. (2011). Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats , India. *Journal of Ethnopharmacology*, 134(3):851–864.

Badarinath, A. V, Rao, K. M., Chetty, C. M. S., Ramkanth, S., Rajan, T. V. S. and Gnanaprakash, K. (2010). A review on in-vitro antioxidant methods: comparisions, correlations and considerations. *International Journal of PharmTech Research*, 2(2):1276–1285.

Bakkiyaraj, S. and Pandiyaraj, S. (2011). Evaluation of potential antimicrobial activity of some medicinal plants against common food-borne pathogenic microorganism. *International Journal of Pharma and Bio Sciences*, 2(2).

Barbosa-Filho, J. M., Piuvezam, M. R., Moura, M. D., Silva, M. S., Lima, K. V. B., da-Cunha, E. V. L., Fechine, I. M. and Takemura, O. S. (2006). Antiinflammatory activity of alkaloids: A twenty-century review. *Revista Brasileira de Farmacognosia*, 16(1):109–139.

Belguidoum, M., Dendougui, H. and Kendour, Z. (2015). In vitro antioxidant properties and phenolic contents of *Zygophyllum album* L. from Algeria. *Journal of Chemical and Pharmaceutical Research*, 7(1).

Benzie, I. F. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Analytical biochemistry*, 239(1):70–76.

Bouch, C. H. (2004). *Laportea ovalifolia* (Schumach.) Chew. Vegetable/Legumes Grubben GJH, and Denton AO,(eds) Porta Press, London.

Braca, A., De Tommasi, N., Di Bari, L., Pizza, C., Politi, M. and Morelli, I. (2001). Antioxidant principles from bauhinia t arapotensis. *Journal of natural products*, 64(7):892–895.

British Pharmacopoeia (2000) 5th edition Formulated preparation: specific monograph Vol. III.

Burke, A., Smyth, E. and FitzGerald, G. A. (2006). Analgesic-antipyretic agents; pharmacotherapy of gout. In: Brunton, L. L. (Ed.) Goodman and Gilman's The Pharmacological Basis Of Therapeutics. McGraw-Hill Medical Publishing Division, New York.

Buss, A. D., Cox, B. and Waigh, R. D. (2003). Natural products as leads for new pharmaceuticals, in: Burger's Medicinal Chemistry and Drug Discovery. John Wiley & Sons, Inc. (pp. 847–900).

Buzzini, P., Arapitsas, P., Goretti, M., Branda, E., Turchetti, B., Pinelli, P., Ieri, F. and Romani, A. (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini reviews in medicinal chemistry*, 8(12):1179–1187.

Calixto, J. B. (2005). Twenty-five years of research on medicinal plants in Latin America: a personal view. *Journal of ethnopharmacology*, 100(1):131–134.

Chew, W. L. (1969). Monograph of Laportea (Urticaceae). *Gard Bull*.

Coates, A., Hu, Y., Bax, R. and Page, C. (2002). The future challenges facing the development of new antimicrobial drugs. *Nature Reviews Drug Discovery*, 1(11):895–910.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G. A., Uzunov, D., Tubaro, A., Menichini, F. and Della Loggia, R. (2008). In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. *Journal of Ethnopharmacology*, 116(1):144–151.

Cos, P., Vlietinck, A. J., Berghe, D. Vanden and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-ofconcept'. *Journal of ethnopharmacology*, 106(3):290–302.

Cragg, G. M. and Newman, D. J. (2013). Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830(6):3670–3695.

Cromartie, W. J., Craddock, J. G., Schwab, J. H., Anderle, S. K. and Yang, C.-H. (1977). Arthritis in rats after systemic injection of streptococcal cells or cell walls. *The Journal of experimental medicine*, 146(6):1585–1602.

Cui, K., Luo, X., Xu, K. and Murthy, M. R. V. (2004). Role of oxidative stress in neurodegeneration: Recent developments in assay methods for oxidative stress and nutraceutical antioxidants. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 28(5):771–799.

Chung, T. Y., Peplow, P. V and Baxter, G. D. (2010). Laser photobiomodulation of wound healing in diabetic and non-diabetic mice: Effects in splinted and unsplinted wounds. *Photomedicine and laser surgery*, 28(2):251–261.

Decker, T. and Lohmann-Matthes, M. L. (1988). A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *Journal of Immunological Methods*, 115(1):61–69.

Devienne, K. F. and Raddi, M. S. G. (2002). Screening for antimicrobial activity of natural products using a microplate photometer. *Brazilian Journal of Microbiology*, 33(2):166–168.

Di Rosa, M. (1972). Biological properties of carrageenan. *Journal of Pharmacy and Pharmacology*, 24(2):89–102.

Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest Journal*, 118(2):503–508.

Dokosi, O. B. (1998). Herbs of Ghana. Ghana Universities Press.

Döll-Boscardin, P. M., Sartoratto, A., Sales Maia, B. H. L. de N., Padilha de Paula, J., Nakashima, T., Farago, P. V. and Kanunfre, C. C. (2012). In vitro cytotoxic potential of essential oils of *Eucalyptus benthamii* and its related terpenes on tumor cell lines. *Evidence-Based Complementary and Alternative Medicine*, 2012.

Dorsett-Martin, W. A. and Wysocki, A. B. (2008). 65 Rat Models of Skin Wound Healing. *Models For Biomedical Research*:631.

Dostert, C., Pétrilli, V., Van Bruggen, R., Steele, C., Mossman, B. T. and Tschopp, J. (2008). Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science*, 320(5876):674–677.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, 4(7):685– 688.

Ekpo, M., Mbagwu, H., Jackson, C. and Eno, M. (2011). Antimicrobial and wound healing activities of *Centrosema pubescens* (Leguminosae). *Journal* of *Pharmacy* and *Clinical Sciences*, 1:1–6.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta medica*, 64(8):711–713.

Essiett, U. A., Edet, N. I., Bala, D. N. (2011). Phytochemical and physicochemical analysis of the leaves of *Laportea aestuans* (Linn.) Chew and *Laportea ovalifolia* (Schumach.) Chew (male and female). *Asian Journal of Plant Science and Research*, 1(2):35–42.

Etukudo, I. (2003). Ethnobotany. Conventional and traditional uses of plants. *The Verdict press, Uyo*, 1:191.

Fabricant, D. S. and Farnsworth, N. R. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental health perspectives*, 109(1):69–75.

Fabry, W., Okemo, P. O. and Ansorg, R. (1998). Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology*, 60(1):79–84.

Farber, J. L. (1994). Mechanisms of cell injury by activated oxygen species. *Environmental health perspectives*, 102(10):17.

Fiori, G. M. L., Fachin, A. L., Correa, V. S. C., Bertoni, B. W., Giuliatti, S., Amui, S. F., de Castro França, S. and Pereira, A. M. S. (2013). Antimicrobial activity and rates of tannins in *Stryphnodendron adstringens* Mart. accessions collected in the Brazilian Cerrado. *American Journal of Plant Sciences*, 4(11):2193.

Fleischer, T. C., Ameade, E. P. K., Mensah, M. L. K. and Sawer, I. K. (2003). Antimicrobial activity of the leaves and seeds of *Bixa orellana*. *Fitoterapia*, 74(1):136–138.

Frei, B., Stocker, R. and Ames, B. N. (1988). Antioxidant defenses and lipid peroxidation in human blood plasma. *Proceedings of the National Academy of Sciences*, 85(24):9748–9752.

Gal, P., Kilik, R., Mokry, M., Vidinsky, B., Vasilenko, T., Mozes, S., Bobrov, N., Tomori, Z., Bober, J. and Lenhardt, L. (2008). Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semi-quantitative and quantitative histological assessments. *Veterinarni Medicina*, 53(12):652–659.

Gbedema, S. Y., Emelia, K., Francis, A., Kofi, A. and Eric, W. (2010). Wound healing properties and kill kinetics of *Clerodendron splendens* G. Don, a Ghanaian wound healing plant. *Pharmacognosy Research*, 2(2):63–68.

Gibbons, S. (2004). Anti-staphylococcal plant natural products. *Natural product reports*, 21(2):263–277.

Gomes, N. G. M., Campos, M. G., Órfão, J. M. C. and Ribeiro, C. A. F. (2009). Plants with neurobiological activity as potential targets for drug discovery. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33(8):1372–1389.

González ‐Martínez, S. C., Krutovsky, K. V and Neale, D. B. (2006). Forest ‐tree population genomics and adaptive evolution. *New Phytologist*, 170(2):227–238.

Gosain, A. and DiPietro, L. A. (2004). Aging and Wound Healing. *World Journal of Surgery*, 28(3):321–326.

Gottrup, F., Ågren, M. S. and Karlsmark, T. (2000). Models for use in wound healing research: A survey focusing on *in vitro* and *in vivo* adult soft tissue. *Repair and Regeneration*, 8(2):83–96.

Guo, S. and DiPietro, L. A. (2010). Factors affecting wound healing. *Journal of Dental Research*, 89(3):219–229.

Graham, D. J. (2006). COX-2 inhibitors, other NSAIDs, and cardiovascular risk: the seduction of common sense. *Jama*, 296(13):1653–1656.

Guardia, T., Rotelli, A. E., Juarez, A. O. & Pelzer, L. E. (2001). Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Il farmaco*, 56(9):683–687.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular aspects of Medicine*, 27(1):1–93.

Habif, T. P. (1996). Dermatologic surgical procedures. *Clinic Dermatology: A Color Guide to Diagnosis and Therapy*, 3:809–810.

Harbarth, S., Nobr, V. and Pittet, D. (2007). Does antibiotic selection impact patient outcome? *Clinical infectious diseases*, 44(1):87–93.

Higgs, G. A. (1989). Use of implanted sponges to study the acute inflammatory response. In: Chang, J. Y. and Lewis, A. J. (Eds.) Pharmacological methods in the control of inflammation. New York: Alan R. Liss, Inc,151–171.

Huang, D., Ou, B. and Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry*, 53(6):1841–1856.

Iffen, T. S. and Usoro, C. A. O. (2010). The effect of ethanolic extract of *Larpotea ovalifolia* plants growing in calabar on antioxidants status of streptozocin induced diabetic rats. *Global Journal of Pharmacology*, 4(1):1–5.

Im Kim, S., Sim, K. H. and Choi, H. Y. (2010). A comparative study of antioxidant activity in some Korean medicinal plant used as food materials. *Molecular and Cellular Toxicology*, 6(3):279–285.

Inatani, R., Nakatani, N. and Fuwa, H. (1983). Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis* L.) and their derivatives. *Agricultural and biological chemistry*, 47(3):521–528.

Irfan, M., Ahmed, S. and Sharma, M. (2014). Antimicrobial activity of terpenoids from *Sphaeranthus indicus*. L. *Asian Journal of Plant Science and Research*, 4(1):1–6.

Issekutz, A. C. and Issekutz, T. B. (1989). Quantitation of blood cell accumulation and vascular responses in inflammatory reactions. In: Chang, J. Y. and Lewis, A. J. (Eds.) Pharmacological methods in the control of inflammation. New York: Alan R. Liss, Inc, (p. 129).

Jain, A., Soni, M., Deb, L., Jain, A., Rout, S. P., Gupta, V. B. and Krishna, K. L. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica* Roxb. leaves. *Journal of Ethnopharmacology*, 115(1):61–66.

Jenke-Kodama, H., Müller, R. and Dittmann, E. (2008). Evolutionary mechanisms underlying secondary metabolite diversity. In: Natural Compounds as Drugs Volume I, (pp. 119–140). Springer.

Kaibara, N., Hotokebuchi, T., Takagishi, K. and Katsuki, I. (1983). Paradoxical effects of cyclosporin A on collagen arthritis in rats. *The Journal of experimental medicine*, 158(6):2007–2015.

Kalcher, K., Svancara, I., Buzuk, M., Vytras, K. and Walcarius, A. (2009). Electrochemical sensors and biosensors based on heterogeneous carbon materials. *Monatshefte für Chemie-Chemical Monthly*, 140(8):861–889.

Kamatou, G. P. P., Van Zyl, R. L., Van Vuuren, S. F., Viljoen, A. M., CristinaFigueiredo, A., Barroso, J. G., Pedro, L. G. and Tilney, P. M. (2006). Chemical composition, leaf trichome types and biological activities of the essential oils of four related Salvia species indigenous to southern Africa. *The Journal of essential oil research*, 18:72–79.

Kane, D. P. (2007). Surgical repair in advanced wound caring. In: Chronic wound care: A clinical source book for healthcare professionals. 4th ed. Malvern, PA: HMP Communications:363–372.

Kaur, G., Hamid, H., Ali, A., Alam, M. S. and Athar, M. (2004). Antiinflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. *Journal of ethnopharmacology*, 90(2):285–292.

Keweloh, H., Heipieper, H. J. and Rehm, H. J. (1989). Protection of bacteria against toxicity of phenol by immobilization in calcium alginate. *Applied Microbiology and Biotechnology*, 31(4):383–389.

Khalaf, N. A., Shakya, A. K., Al-Othman, A., El-Agbar, Z. and Farah, H. (2008). Antioxidant activity of some common plants. *Turkish Journal of Biology*, 32(11):51–56.

Khan, R. A., Khan, M. R., Sahreen, S. and Ahmed, M. (2012). Assessment of flavonoids contents and in vitro antioxidant activity of *Launaea procumbens*. *Chemistry Central Journal*, 6(1):43.

Koleckar, V., Kubikova, K., Rehakova, Z., Kuca, K., Jun, D., Jahodar, L. and Opletal, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini reviews in medicinal chemistry*, 8(5):436–447.

Krasner, D., Rodeheaver, G. T. and Sibbald, R. G. (2007). Chronic wound care: A clinical source book for healthcare professionals, 4th ed. Malvern, PA, HMP Communications.

Krishnan, P. (2006). The scientific study of herbal wound healing therapies: Current state of play. *Current Anaesthesia and Critical Care*, 17(1):21–27.

Kubmarawa, D., Khan, M. E., Punah, A. M. and Hassan, M. (2008). Phytochemical screening and antimicrobial efficacy of extracts from *Khaya senegalensis* against human pathogenic bacteria. *African Journal of Biotechnology*, 7(24).

Kumar, V., Abbas, A. K., Fausto, N. and Aster, J. C. (2010). Robbins and Cotran Pathological Basis of Disease. 8th edn. Saunders Elsevier.

Kumari, S. T. K., Lincy, M. P., Muthukumarasamy, S. and Mohan, V. R. (2012). Anti-inflammatory activity of *Sarcostemma secamone* (I) Bennet whole plant against carrageenan induced paw edema. *Bioscience Discovery*, 3(3):288–291.

Laguerre, M., Lecomte, J. and Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in lipid research*, 46(5):244–282.

Lambert, P. A. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. *Journal of the Royal Society of Medicine*, 95(41):22–26.

Larsen, G. L. and Henson, P. M. (1983). Mediators of inflammation. *Annual review of immunology*, 1(1):335–359.

Lazarus, G. S., Cooper, D. M., Knighton, D. R., Margolis, D. J., Pecoraro, R. E., Rodeheaver, G. and Robson, M. C. (1994). Definitions and guidelines for assessment of wounds and evaluation of healing. *Archives of Dermatology*, 130(4):489–493.

Letouzey, R. (1968). Urticaceae. *Flore du Cameroun*, 8:67–216.

Levy, S. B. (2005). Antibiotic resistance the problem intensifies. *Advanced drug delivery reviews*, 57(10):1446–1450.

Levy, S. B. and Marshall, B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature medicine*, 10:S122–S129.

Lourens, A. C. U., Reddy, D., Başer, K. H. C., Viljoen, A. M. and Van Vuuren, S. F. (2004). In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. *Journal of Ethnopharmacology*, 95(2):253–258.

Lupu, A. R. and Popescu, T. (2013). The noncellular reduction of MTT tetrazolium salt by TiO 2 nanoparticles and its implications for cytotoxicity assays. *Toxicology in Vitro*, 27(5):1445–1450.

Maganha, E. G., Da Costa-Halmenschlager, R., Rosa, R. M., Henriques, J. A. P., De Paula-Ramos, A. L. L. and Saffi, J. (2010). Pharmacological evidences for the extracts and secondary metabolites from plants of the genus Hibiscus. *Food Chemistry*, 118(1):1–10.

Mahmood, A. A., Sidik, K. and Salmah, I. (2005). Wound healing activity of *Carica papaya* L. aqueous leaf extract in rats. *International Journal of Molecular Medicine and Advance Sciences*, 1(4):398–401.

Marchal, E., Uddin, M. I., Smithen, D. A., Hawco, C. L. A., Lanteigne, M., Overy, D. P., Kerr, R. G. and Thompson, A. (2013). Antimicrobial activity of non-natural prodigiosenes. *RSC Advances*, 3(45):22967–22971.

Margret, R. J., Kumaresan, S. and Ravikumar, S. (2009). A preliminary study on the anti-inflammatory activity of methanol extract of *Ulva lactuca* in rat. *Journal of Environmental Biology*, 30(5):899-902

Martin, P. and Leibovich, S. J. (2005). Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends in cell biology*, 15(11):599–607.

Masih, N. G. and Singh, B. S. (2012). Phytochemical screening of some plants used in herbal based cosmetic preparations, in: Chemistry of Phytopotentials: Health, Energy and Environmental Perspectives, (pp. 111–112). Springer.

Matsumoto, M., Ishida, K., Konagai, A., Maebashi, K. and Asaoka, T. (2002). Strong antifungal activity of SS750, a new triazole derivative, is based on its selective binding affinity to cytochrome P450 of fungi. *Antimicrobial agents and chemotherapy*, 46(2):308–314.

Mazzola, P. G., Jozala, A. F., Novaes, L. C. de L., Moriel, P. and Penna, T. C. V. (2009). Minimal inhibitory concentration (MIC) determination of disinfectant and/or sterilizing agents. *Brazilian Journal of Pharmaceutical Sciences*, 45(2):241–248.

Mbagwu, H. O., Anene, R. A. and Adeyemi, O. O. (2006). Analgesic, antipyretic and anti-inflammatory properties of *Mezoneuron benthamianum* Baill (Caesalpiniaceae). *Nigerian quarterly journal of hospital medicine*, 17(1):35–41.

McGuckin, M., Goldman, R., Bolton, L. and Salcido, R. (2003). The Clinical Relevance of Microbiology in Acute and Chronic Wounds. *Advances in Skin and Wound Care*, 16(1):12–23.

Mensah, A. Y., Houghton, P. J., Fleischer, T. C., Mensah, M., Agyare, C., Annan, K. and Dickson, R. (2004). Antimicrobial and antioxidant aspects of *Commelina diffusa*, a Ghanaian plant used traditionally for wound healing. *Journal of Pharmacy and Pharmacology*, (pp. 79–79).

Meszaros, A. J., Reichner, J. S. and Albina, J. E. (2000). Macrophage-induced neutrophil apoptosis. *The Journal of Immunology*, 165(1):435–441.

Momo, C. E. N., Oben, J. E., Tazoo, D. and Dongo, E. (2006). Antidiabetic and hypolipidemic effects of *Laportea ovalifolia* (Urticaceae) in Alloxan induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines*, 3(1):36 – 43.

Morris, C. J. (2003). Carrageenan-induced paw edema in the rat and mouse. *Methods in molecular biology*, 225:115–21.

Morton, J. J. and Malone, M. H. (1972). Evaluation of vulneray activity by an open wound procedure in rats. *Archives Internationales de Pharmacodynamie et de Therapie*, 196(1):117–126.

Mosser, D. M. and Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature reviews immunology*, 8(12):958–969.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival:

application to proliferation and cytotoxicity assays. *Journal of immunological methods*, 65(1):55–63.

Mshana, N. R. (2000). Traditional medicine and pharmacopoeia: Contribution to the revision of ethnobotanical and floristic studies in Ghana. Organization of African Unity/Scientific, Technical and Research Commission.

Murthy, M. R. V. (2001). Coenzyme-Q and related isoprenoid compounds: Biosynthesis, regulation, functions and biomedical implications. In: Ebadi, M., Marwah, J., Chopra, R. (Eds.), Mitochondrial Ubiquinone (Coenzyme Q-10): biochemical, functional, medical, and therapeutic aspects. Prominent Press.

Murugan, T., Wins, J. A. and Murugan, M. (2013). Antimicrobial activity and phytochemical constituents of leaf extracts of *Cassia auriculata*. *Indian Journal of Pharmaceutical Sciences*, 75(1):122.

Nagori, B. P. and Solanki, R. (2011). Role of medicinal plants in wound healing. *Research Journal of Medicinal Plant*, 5(4):392–405.

Narayanaswamy, N. and Balakrishnan, K. P. (2011). Evaluation of some medicinal plants for their antioxidant properties. *International Journal of PharmTech Research*, 3(1):381–385.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C. and Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4):247–256.

Navarro, V. and Delgado, G. (1999). Two antimicrobial alkaloids from *Bocconia arborea*. *Journal of Ethnopharmacology*, 66(2):223–226.

Ncube, N. S., Afolayan, A. J. and Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *African Journal of Biotechnology*, 7(12).

Niles, A. L., Moravec, R. A. and Riss, T. L. (2008). Update on *in vitro* cytotoxicity assays for drug development. *Expert Opinion on Drug Discovery*, 3(6):655–669.

Nweze, E. I., Okafor, J. I. and Njoku, O. (2004). Antimicrobial activities of methanolic extracts of *Trema guniesnsis* (Schum and Thom) and *Morinda lucida* Benth used in Nigerian Herbal Medical Practices. *Journal of Biological Research and Biotechnology*, 2(1):39–46.

Obiri, D., Osafo, N. and Dei-Anane, S. (2013). Anti-allergic, anti-inflammatory and analgesic actions of stem bark extract of *Lannea welwitschii* (Anarcadiaceae) in mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(4):217–222.

Obiri, D. D. and Osafo, N. (2013). Aqueous ethanol extract of the fruit of *Xylopia aethiopica* (Annonaceae) exhibits anti-anaphylactic and anti-inflammatory actions in mice. *Journal of Ethnopharmacology*, 148(3):940–945.

Ofori-Kwakye, K., Kwapong, A. A. and Bayor, M. T. (2011). Wound healing potential of methanol extract of *Spathodea campanulata* stem bark forumlated into a topical preparation. *African Journal of Traditional, Complementary and Alternative Medicines*, 8(3):218–223.

Okwulehie, I. C. and Akanwa, F. E. (2013). Antimicrobial Activity of Ethanol Extract of Four Indigenous Plants from South Eastern Nigeria. *ARPN Journal of Science and Technology*, 3(4):350–355.

Oluwatuyi, M., Kaatz, G. W. and Gibbons, S. (2004). Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. *Phytochemistry*, 65(24):3249– 3254.

Oryan, A., Naeini, A. T., Nikahval, B. and Gorjian, E. (2010). Effect of aqueous extract of *Aloe vera* on experimental cutaneous wound healing in rat. *Veterinarski arhiv*, 80(4):509–522.

Ozaki, Y. (1990). Antiinflammatory effect of *Curcuma xanthorrhiza* Roxb, and its active principles. *Chemical and Pharmaceutical Bulletin*, 38(4):1045–1048.

Patel, V. R., Patel, P. R. and Kajal, S. S. (2010). Antioxidant activity of some medicinal plants of western region of India. *Advances in Biological Research*, 4(1):23–26.

Pisoschi, A. M. and Negulescu, G. P. (2011). Methods for total antioxidant activity determination: A review. *Biochemistry and Analytical Biochemistry*, 1(1).

Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2):337–341.

Prior, R. L., Hoang, H. A., Gu, L., Wu, X., Bacchiocca, M., Howard, L., HampschWoodill, M., Huang, D., Ou, B. and Jacob, R. (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *Journal of Agricultural and Food Chemistry*, 51(11):3273–3279.

Projan, S. J. (2003). Why is big Pharma getting out of antibacterial drug discovery? *Current Opinion in Microbiology*, 6(5):427–430.

Qader, S. W., Abdulla, M. A., Chua, L. S., Najim, N., Zain, M. M. and Hamdan, S. (2011). Antioxidant, Total Phenolic Content and Cytotoxicity Evaluation of Selected Malaysian Plants. *Molecules*, 16(4):3433–3443.

Ramos, D. F., Leitão, G. G., Costa, F. das N., Abreu, L., Villarreal, J. V., Leitão, S. G., Saidy-Fernández, S. L. and da Silva, P. E. A. (2008). Investigation of the

antimycobacterial activity of 36 plant extracts from the Brazilian Atlantic Forest. *Revista Brasileira de Ciências Farmacêuticas*, 44(4):669–674.

Rang, H. P. (2007). Rang and Dale's Pharmacology 6th edition. *Edinburgh: Churchill Livingstone*.

Ranjan, S., Bhavya, K., Muntaj, S., Basumata, G. and Matcha, R. (2012). Synergistic effect of some medicinal plants and antibiotics against few pathogenic bacteria. *International Journal of Biological and Pharmaceutical Research*, 3(8):1000–1004.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, 20(7):933–956.

Rios, J. L. and Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100(1):80–84.

Rizki, T. M. and Rizki, R. M. (1992). Lamellocyte differentiation in Drosophila larvae parasitized by Leptopilina. *Developmental and Comparative Immunology*, 16(2):103–110.

Robert, A. and Nezamis, J. E. (1957). The granuloma pouch as a routine assay for antiphlogistic compounds. *Acta Endocrinologica*, 25(1):105–112.

Rock, K. L. and Kono, H. (2008). The inflammatory response to cell death. *Annual Review of Pathology*, 3:99–126.

Rotelli, A. E., Guardia, T., Juárez, A. O., De la Rocha, N. E. and Pelzer, L. E. (2003). Comparative study of flavonoids in experimental models of inflammation. *Pharmacological Research*, 48(6):601–606.

Schmelzer, G. H. and Gurib-Fakim, A. (2008). Plant resources of Tropical Africa (program). Medicinal Plants 1, PROTA Foundation, Wageningen, Netherlands.

Schmid-Schönbein, G. W. (2006). Analysis of inflammation. *Annual Review of Biomedical Engineering*, 8:93–131.

Scorzoni, L., Benaducci, T., Almeida, A. M. F., Silva, D. H. S., Bolzani, V. da S. and Gianinni, M. J. S. M. (2007a). The use of standard methodology for determination of antifungal activity of natural products against medical yeasts Candida sp and Cryptococcus sp. *Brazilian Journal of Microbiology*, 38(3):391– 397.

Scorzoni, L., Benaducci, T., Almeida, A., Silva, D., Bolzani, V. S., Giannini, M. and Soares, M. J. (2007b). Comparative study of disk diffusion and microdilution methods for evaluation of antifungal activity of natural compounds against medical yeasts Candida spp and Cryptococcus sp. *Revista de Ciências Farmacêuticas Básica e Aplicada*, 28(1):25–34.

Selye, H. (1953). On the mechanism through which hydrocortisone affects the resistance of tissues to injury: An experimental study with the granuloma pouch technique. *Journal of the American Medical Association*, 152(13):1207–1213.

Shailasree, S., Ruma, K., Kini, K. R., Niranjana, S. R. and Prakash, H. S. (2012). Reveiw Article: Potential anti-inflammatory bioactives from medicinal plants of Western Ghats, India. *Pharmacognosy Communications*, 2(2):2–12.

Sharma, U. K., Singh, A., Sharma, U., Kumar, M., Rai, D. and Agrahari, A. (2010). Wound healing activity of *Kigelia pinnata* bark extract. *Asian Journal of Pharmaceutical and Clinical Research*, 3(4):73–75.

Shetty, S., Udupa, S. and Udupa, L. (2008). Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. *Evidence-Based Complementary and Alternative Medicine*, 5(1):95–101.

Sibanda, T. and Okoh, A. I. (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. *African Journal of Biotechnology*, 6(25).

Singh, G. and Kumar, P. (2012). Antibacterial potential of alkaloids of *Withania somnifera* L. and *Euphorbia hirta* L. *International Journal of Pharmacy and Pharmaceutical Sciencesb*, 4(1).

Singh, A., Malhotra, S. and Subban, R. (2008). Anti-inflammatory and analgesic agents from indian medicinal plants. *Bangladesh Journal of Pharmacology*, 3(1):57–72.

Singh, M., Govindarajan, R., Nath, V., Rawat, A. K. S. and Mehrotra, S. (2006). Antimicrobial, wound healing and antioxidant activity of *Plagiochasma appendiculatum* Lehm. et Lind. *Journal of Ethnopharmacology*, 107(1):67–72.

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., Simonič, M. and Knez, Ţ. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food chemistry*, 89(2):191–198.

Slinkard, K. and Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28(1):49–55.

Smith, S. M., Wunder, M. B., Norris, D. A. and Shellman, Y. G. (2011). A simple protocol for using a LDH-based cytotoxicity assay to assess the effects of death and growth inhibition at the same time. *PloS one*, 6(11):e26908.

Socha, R., Juszczak, L., Pietrzyk, S. and Fortuna, T. (2009). Antioxidant activity and phenolic composition of herbhoneys. *Food Chemistry*, 113(2):568–574.

Sofowora, A. (1993). Recent trends in research into African medicinal plants. *Journal of Ethnopharmacology*, 38(2):197–208.

Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*, 2(3):365–372.

Stackpole, D. J., Vaillancourt, R. E., Alves, A., Rodrigues, J. and Potts, B. M. (2011). Genetic variation in the chemical components of *Eucalyptus globulus* wood. *G3: Genes, Genomes, Genetics*, 1(2):151–159.

Stadelmann, W. K., Digenis, A. G. and Tobin, G. R. (1998). Physiology and healing dynamics of chronic cutaneous wounds. *The American Journal of Surgery*, 176(2):26S–38S.

Stermitz, F. R., Tawara-Matsuda, J., Lorenz, P., Mueller, P., Zenewicz, L. and Lewis, K. (2000). 5'-Methoxyhydnocarpin-D and Pheophorbide A: Berberis species components that potentiate berberine growth inhibition of resistant *Staphylococcus aureus*. *Journal of Natural Products*, 63(8):1146–1149.

Suguna, L., Singh, S., Sivakumar, P., Sampath, P. and Chandrakasan, G. (2002). Influence of *Terminalia chebula* on dermal wound healing in rats. *Phytotherapy Research*, 16(3):227–231.

Süntar, I., Koca, U., Keleş, H. and Akkol, E. K. (2009). Wound Healing Activity of *Rubus sanctus* Schreber (Rosaceae): Preclinical study in animal models. *Evidence Based Complementary and AlternativeMedicine*, 2011:37.

Susanti, D., Sirat, H. M., Ahmad, F., Ali, R. M., Aimi, N. and Kitajima, M. (2007). Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chemistry*, 103(3):710–716.

Tabuti, J. R. S., Lye, K. A. and Dhillion, S. S. (2003). Traditional herbal drugs of Bulamogi, Uganda: Plants, use and administration. *Journal of Ethnopharmacology*, 88(1):19–44.

Talekar, Y. P., Das, B., Paul, T., Talekar, D., Apte, K. G. and Parab, P. B. (2012). Evaluation of wound healing potential of aqueous and ethanolic extracts of *tridax procumbens* linn. In wistar rats. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4):141–145.

Tang, T., Yin, L., Yang, J. and Shan, G. (2007). Emodin, an anthraquinone derivative from *Rheum officinale* Baill, enhances cutaneous wound healing in rats. *European Journal of Pharmacology*, 567(3):177–185.

Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *The American Journal Of Medicine*, 119(6):S3–S10.

Thomazzi, S. M., Silva, C. B., Silveira, D. C. R., Vasconcellos, C. L. C., Lira, A. F., Cambui, E. V. F., Estevam, C. S. and Antoniolli, A. R. (2010). Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). *Journal of Ethnopharmacology*, 127(2):451–456.

Tistaert, C., Dejaegher, B., Chataigné, G., Rivière, C., Hoai, N. N., Van, M. C., Quetin-Leclercq, J. and Vander Heyden, Y. (2012). Potential antioxidant compounds in Mallotus species fingerprints. Part II: fingerprint alignment, data analysis and peak identification. *Analytica Chimica Acta*, 721:35–43.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 1(1):98–106.

Trease, G. E. and Evans, W. C. (2002). Pharmacognosy. Saunders, Elsevier, Amsterdam, Netherlands, 36:51.

Trentham, D. E., Townes, A. S. and Kang, A. H. (1977). Autoimmunity to type II collagen an experimental model of arthritis. *The Journal of Experimental Medicine*, 146(3):857–868.

Udegbunam, S. O., Udegbunam, R. I., Muogbo, C. C., Anyanwu, M. U. and Nwaehujor, C. O. (2014). Wound healing and antibacterial properties of methanolic extract of Pupalia lappacea Juss in rats. *BMC complementary and alternative medicine*, 14(1):157.

Udupa, A. L., Kulkarni, D. R. and Udupa, S. L. (1995). Effect of *Tridax procumbens* extracts on wound healing. *Pharmaceutical Biology*, 33(1):37–40.

Umeh, E. U., Oluma, H. O. A. and Igoli, J. O. (2005). Research communication: Antibacterial screening of four local plants using an indicator-based microdilution technique. *Africa Journal of Traditional, Complementary and Alternative Medicines*, 2(3):238 – 243.

Usman, J. G., Sodipo, O. A. and Sandabe, U. K. (2014). Phytochemical screening and acute toxicity study of *Cucumis metuliferus* E. Mey. Ex. Naudin fruit extract in cockerels. *International Journal of Phytomedicine*, 6(2):243.

Vaghasiya, Y., Dave, R. and Chanda, S. (2011). Phytochemical analysis of some medicinal plants from western region of India. *Research Journal of Medicinal Plant*, 5(5):567–576.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry and Cell Biology*, 39(1):44–84.

Vinegar, R., Truax, J. F., Selph, J. L., Johnston, P. R., Venable, A. L. and McKenzie, K. K. (1987). Pathway to carrageenan-induced inflammation in the hind limb of the rat, in: Federation proceedings, (pp. 118–126).

Weichman, B. M. (1989). Rat adjuvant arthritis: A model of chronic inflammation. In: Chang, J. Y. and Lewis, A. J. (Eds.) Pharmacological methods in the control of inflammation. New York: Alan R. Liss, Inc,363–380.

Whicher, J. T., Thompson, D., Billingham, M. E. J. and Kitchen, E. A. (1989). Acute Phase Proteins. In: Chang, J. Y. and Lewis, A. J. (Eds.) Pharmacological methods in the control of inflammation. New York: Alan R. Liss, Inc.

Wiegand, I., Hilpert, K. and Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, 3(2):163–175.

Winter, C. A. and Porter, C. C. (1957). Effect of alterations in side chain upon anti - inflammatory and liver glycogen activities of hydrocortisone esters. *Journal of the American Pharmaceutical Association*, 46(9):515–519.

Winter, C. A., Risley, E. A. and Nuss, G. W. (1962). Carrageenin-induced edema in hind paw of the rat as an assay for antiinflammatory drugs. *Experimental Biology and Medicine*, 111(3):544–547.

Woode, E. and Abotsi, W. K. M. (2011). Antinociceptive effect of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt.(Phytolaccaceae). *Journal of Pharmacy and Bioallied Sciences*, 3(3):384.

Woode, E., Abotsi, W. K. M. and Mensah, A. Y. (2011). Anxiolytic-and antidepressant-like effects of an ethanolic extract of the aerial parts of *Hilleria latifolia* (Lam.) H. Walt. in mice. *Journal of Natural Pharmaceuticals*, 2(2).

Wu, S. J. and Ng, L. T. (2008). Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn. var. abbreviata Ser.) in Taiwan. *LWT-Food Science and Technology*, 41(2):323–330.

Xu, H. X. and Lee, S. F. (2001). Activity of plant flavonoids against antibioticresistant bacteria. *Phytotherapy Research*, 15(1):39–43.

Yadav, K. C. H., Kumar, J. R., Basha, S. I., Deshmukh, G. R., Gujjula, R. and Santhamma, B. (2012). Wound healing activity of topical application of *Aloe vera* gel in experimental animal models. *International Journal of Pharmacy and Biological Sciences*, 3(2):63–72.

Yang, Y. P., Shih, B. L. and Liu, H. Y. (1996). Urticaceae. *Flora of Taiwan*, 2:197–257.

Yin, H., Xu, L. and Porter, N. A. (2011). Free radical lipid peroxidation: Mechanisms and analysis. *Chemical Reviews*, 111(10):5944–5972.

Zheng, W. and Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food chemistry*, 49(11):5165–5170.

APPENDICE

APPENDIX 1: Composition and preparation of microbial media

A1.1 Nutrient agar (Oxoid, Basingstoke, UK)

Table 1.1: Composition of Nutrient agar

Composition	Quantity (g)
Lab-lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

A quantity of 28.0 g Nutrient agar powder was weighed into a beaker. Eight hundred millilitres of sterile distilled water was added and heated at 100^{o}C until it begins to froth. The resulting mixture was then made up to 1 L with sterile distilled water and transferred in 20 mL quantities into glass test tubes. The mixture was then sterilised in an autoclave at 121^{o}C for 15 minutes.

A1.2 Nutrient broth (Oxoid, Basingstoke, UK)

Table 1.2: Composition of Nutrient broth

Composition	Quantity (g)
Lab-lemco powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0

A quantity of 13.0 g Nutrient broth powder was weighed into a beaker. Four hundred millilitres of sterile distilled water was added and stirred to dissolve. Sterile distilled water was added to prouce 1 L solution. Ten millilitres of the solution was transferred into glass test tubes and sterilized at 121^{o}C for 15 minutes in an autoclave.

A1.3 MacConkey agar (Oxoid, Basingstoke, UK)

Table 1.3: Composition of MacConkey agar

Composition	Quantity (g)
Peptone	20.00
Lactose	10.00
Bile salts	1.50
Sodium chloride	5.00
Neutral red	0.03
Crystal violet	0.001

Agar	15.0

A quantity of 51.5 g MacConkey agar powder was weighed into a beaker and suspended in 800 mL sterile distilled water. The mixture was boiled, made up to a volume of 1 L and sterilized in an autoclave at 121^{o}C for 15 minutes.

A1.4 Mannitol salt agar (Oxoid, Basingstoke, UK)

Table 1.4: Composition of Mannitol salt agar

Composition	Quantity (g)
Lab-lemco powder	1.00
Peptone	10.00
Mannitol	10.00
Sodium chloride	75.00
Phenol Red	0.025
Agar	15.0

A quantity of 111.0 g Mannitol salt agar powder was weighed into a beaker and suspended in 800 mL of sterile distilled water. The mixture was boiled, made up to 1 L with distill water and sterilized in an autoclave at 121^{o}C for 15 min.

A1.5 Cetrimide agar (Oxoid, Basingstoke, UK)

Table 1.5: Composition of Cetrimide agar

Composition	Quantity (g)
Gelatin peptone	20.00
Magnesium chloride	1.40
Potassium sulphate	10.00
Cetrimide	0.30
Agar	13.60

A quantity of 45.3 g Cetrimide agar powder was weighed into a beaker and suspended in 800 mL sterile distilled water. The mixture was boiled, made up to 1 L and sterilized in an autoclave at 121^{o}C for 15 min.

A1.6 Bismuth sulphite agar (Oxoid, Basingstoke, UK)

Table 1.6: Composition of Bismuth sulphite agar

Composition	Quantity (g)
Peptone	5.00
Lab-lemco powder	5.00
Glucose	5.00
Di-sodium phosphate	4.00
Ferrous sulphate	0.30

Bismuth sulphite indicator	8.00
Brilliant green	0.02
Agar	12.70

A quantity of 40 g of Bismuth sulphite agar powder was weighed into a beaker and suspended in 800 mL sterile distilled water. The mixture was heated until it began to boil and simmer. The suspension was made up to 1 L whiles heating. The suspension was then transfered into glass test tubes.

A1.7 Potato dextrose agar (Oxoid, Basingstoke, UK)

Table 1.7: Composition of Potato dextrose agar

Composition	Quantity (g)
Potato extract	4.00
Dextrose	20.00
Agar	15.00

A quantity of 39.0 g of potato dextrose agar powder was weighed into a beaker and suspended to 800 mL sterile distilled water. The mixture was boiled, made up to 1 L and sterilized in an autoclave at 121^{o}C for 15 min.

A1.8 Koser's citrate medium (Oxoid, Basingstoke, UK)

Table 1.8: Composition of Koser's citrate medium

Composition	Quantity (g)
Sodium Ammonium Phosphate	1.50
Potassium Dihydrogen Phosphate	1.00
Magnesium sulphate	0.20
Sodium citrate	2.50
Bromothymol blue	0.016

A quantity of 5.2 g of the powder was weighed into a beaker containing 800 mL of sterile distilled water and stirred to dissolve. Sterile distilled water was added to produce a final volume of 1 L. Ten (10) millilitres of the solution was then distributed into glass test tubes and sterilized at 121^{o}C for 15 minutes in an autoclave.

A1.9 Tryptone broth (Oxoid, Basingstoke, UK)

Table1. 9: Composition of Potato dextrose agar

Composition	Quantity (g)
Tryptone	10.00
Sodium chloride	5.00

A quantity of 15.0 g of the powder was weighed into a beaker and dissolved in 800 mL of sterile distilled water. Enough sterile distilled water was added to produce a final voume of 1 L. Ten (10) millilitres of the mixture was then distributed into glass test tubes and sterilized at 121^{o}C for 15 minutes in an autoclave.

A1.10 MRVP medium (Oxoid, Basingstoke, UK)

Table 1.10: Composition of MRVP medium

Composition	Quantity (g)
Peptone	7.00
Glucose	5.00
Phosphate buffer	5.00

A quantity of 17.0 g of the powder was weighed into a beaker and dissolved in 800 mL of sterile distilled water. Enough sterile distilled water was added to produce a final volume of 1 L. Ten (10) millilitres of the mixture was then distributed into glass test tubes and sterilized at 121^{o}C for 15 minutes in an autoclave.

APPENDIX 2: Confirmatory tests on test organisms

Confirmatory test was performed on both the typed and clinical strains used in the study to confirm their identity.

A2.1 *Staphylococcus aureus*

Staphylococcus aureus was identified by culturing a suspension of organisms on mannitol salt agar followed by coagulsae test. The appearance of yellow colonies on the surface of the agar after 24 h incubation at 37ºC indicates the presence of *S. aureus*. Furthermore, the isolated organisms on mannitol salt agar are re-cultured in nutrient broth. A quantity of 0.1 mL of a 24 h broth culture of the isolated colonies was inoculated into nutrient agar containing 10% v/v rabbit blood plasma and incubated for 24 h at 37ºC. The coagulation of the plasma (coagulase positive) confirms the suspension to be *S. aureus*.

A2.2 *Bacillus subtilis*

Starch and casein hydrolysis was used to identify *B. subtilis*. Nutrient agar containing 10% w/v starch was prepared. A loopful of 24 h broth culture of the suspension of organism was streaked at the middle on the surface of nutrient agar containing 10% w/v starch and incubated at 37ºC for 24 h. After incubation the plate was then sprayed with iodine solution. Clear region due to the hydrolysis of starch were seen against a blue black surrounding. The clear zones were indicative of the presence of *B. subtilis*.

A2.3 *Streptococcus pyogenes*

The identification of S. pyogenes was performed with blood agar. Nutrient agar was melted and mixed with about 5% defribinated horse blood and poured into a petri dish. A 24 h broth culture of the organism was placed on the surface of the agar using a straightened loop as spots and incubated at 37 ºC for 24 h. The presence of clear zones around the organism spots made indicates the haemolysis of red blood cells (β-haemolysis) hence the presence of S. pyogenes.

A2.4 *Escherichia coli*

MacConkey agar was seeded with the organisms by streaking and the plate incubated at 37ºC for 24 h. The appearance of red-violet colonies on the surface of the agar after incubation indicates the presence of *E. coli*. The indole and Methy Red-Voges Proskauer (MRVP) tests were used as confirmatory test where this organism is known to be MR positive and VP negative. In the indole test 0.1 mL of the suspension of organism is inoculated in tryptone broth and incubated at 37ºC for 24 h. After incubation Kovac's reagent was added to the tryptone broth. The appearance of a pink/red ring at the interface or meniscus of the broth indicated the presence of *E. coli*. The MRVP test was performed by inoculating a 24 h broth culture of the organisms in MRVP broth.

After incubation the culture was divided into two for the distinct MR and VP tests. In the MR test methyl red was added to the culture and the appearance of pink/red colouration shows that the organism is MR positive. α-naphthol and KOH solutions

were added to the second part of the broth culture for the VP test. The absence of a cherry red colouration indicates that the organism was VP negative.

A2.5 *Pseudomonas aeruginosa*

Two loopfuls of a suspension of the microorganisms was streaked on cetrimide agar and incubated at 37ºC for 24 h incubation. The observation of greenish colonies on the surface of the agar after incubation indicates the presence of *P. aeruginosa*.
Ten (10) milliliters of Koser's citrate medium was inoculated with a loopful of the colonies. The conversion of the green Koser's citrate to blue colouration confirmed the identity of *P. aeruginosa*.

A2.6 *Salmonella typhi*

Bismuth sulphite agar was inoculated with two loopfuls of the microorganisms and incubated at 37ºC for 24 h. Black colonies surrounded by metallic sheen observed within 18-24 hours of incubation indicates the presence of *S. typhi*.

A2.7 *Klebsiella pneumoniae*

Indole, MRVP and catalase tests were used to confirm *K. pneumoniae*. The indole test was performed by inoculating 0.1mL of a 24 h broth culture of the organism in typtone broth and incubating at 37 ºC for 24 h. The absence of a pink/red colour after the addition of Kovac's reagent to the overnight culture indicates the presence of *K. pneumoniae*. MRVP test was performed by inoculating a 24 h broth culture of the organisms in MRVP broth. The culture after incubation was divided into two; one part for the MR test and the other for the VP test. The MR test was performed by the addition of methyl red to the culture; the appearance of yellow colouration indicates that the organism is MR negative. The VP test was performed by the addition of α-naphthol and KOH solutions; the appearance of a cherry red colouration indicates that the organism was VP positive. The catalase test was performed by placing 1 mL of fresh 3 % hydrogen peroxide into a glass test tube and inoculating with about 0.5mL of the bacteria culture. The appearance of air bubbles indicates the presence of catalase enzyme in *K. pneumonia*.

A2.8 *Candida albicans*

A 24 h broth culture of the suspension of organisms was streaked on the surface of potato dextrose agar containing 1 g chloramphenicol and incubated at 25°C for 72 h. Two loopfuls of colonies were streaked on the surface of eosin methylene blue agar. The appearance of white colonies indicated the presence of *C. albicans*.

APPENDIX 3: Effect of sub-inhibitory concentration (5mg/mL) of HLML and LOML on some antibiotics

Table 3.1: MICs of antibiotics only and antibiotics in the presence of sub-inhibitory concentration of extracts

	Test microorganisms

Antibiotics only/ antibiotics + HLML/LOML		*EC*	*BS*	*ST*	*KP*	*SA*	*EF*	*SP*	*PA*
		MIC (µg/mL)							
Amoxy	Only	256	128	128	128	256	>1024	256	128
	HLML	32	32	64	128	32	>1024	>1024	64
	LOML	128	128	256	256	128	>1024	>1024	128
Ampi	Only	128	32	128	128	64	>1024	64	64
	HLML	64	32	32	128	64	>1024	>1024	64
	LOML	128	128	128	256	64	>1024	>1024	>1024
Tetra	Only	16	32	32	64	32	32	32	64
	HLML	32	32	32	16	32	32	32	32
	LOML	128	256	128	128	128	128	128	128
Erythro	Only	32	256	64	128	64	128	64	128
	HLML	512	1024	1024	1024	1024	1024	512	512
	LOML	512	512	512	1024	512	512	1024	1024
Cipro	Only	2	2	4	4	4	4	4	4
	HLML	16	16	16	8	8	8	8	16
	LOML	16	32	32	32	32	16	16	32

APPENDIX 4: Antioxidant activity

A4.1 DPPH free radical scavenging activity Table 3.1: Percentage inhibition

conc (µg/mL)	Vit E			HLML			HLMR			LOML		
15.6	21.9	21.3	21.8	6.5	6.5	6.6	1.6	1.1	1.7	5.8	6.5	5.8
31.3	30.6	30.5	30.4	10.9	10.9	10.4	2.6	3.5	2.7	9.5	10.9	9.5
62.5	56.5	54.9	56.1	21.4	21.4	22.3	9.7	9.8	9.3	17.3	18.5	16.3
125.0	77.8	78.7	78.2	29.2	29.4	30.7	13.7	13.5	16.2	24.8	24.3	24.8
250.0	78.7	78.7	75.5	35.8	37.7	37.3	20.4	20.1	18.5	29.4	27.2	29.4
500.0	76.5	78.0	78.5	36.5	40.7	38.3	21.2	23.3	23.6	37.9	35.5	37.9
1000.0	75.3	79.3	73.0	50.6	50.8	53.8	33.2	33.2	34.7	42.3	41.0	42.3

A4.2 Total phenol content

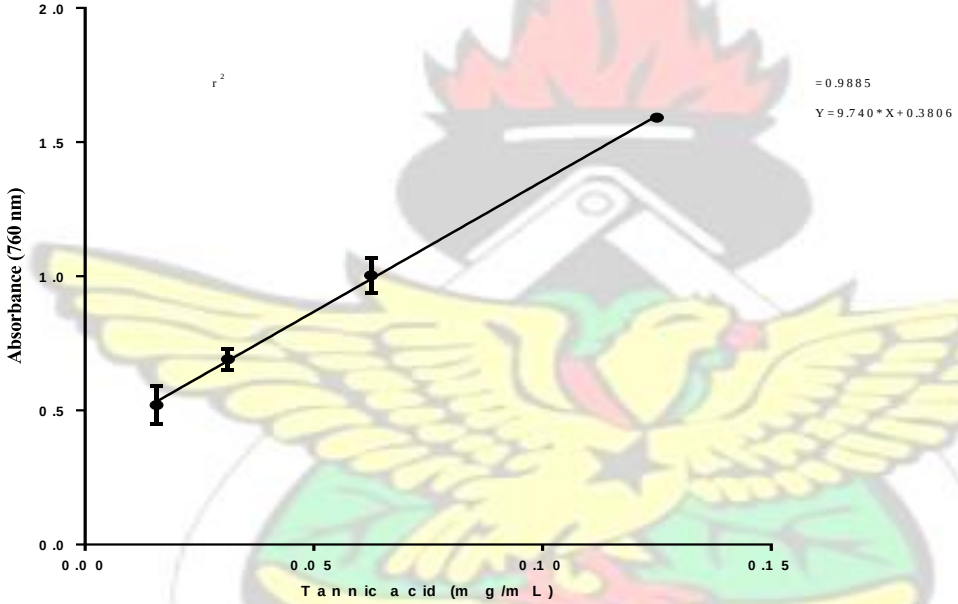

Figure 4.2: Tannic acid calibration curve for total phenol content determination

Table 4.2: Total phenol content of extracts expressed as tannic acid equivalence

Conc (mg/mL)	LML			LOML			HLMR		
10.0	194.91	200.55	198.50	120.06	119.96	124.89	166.57	174.17	186.69
8.0	187.93	191.21	187.21	101.79	101.48	99.73	155.17	154.46	155.07
6.0	167.19	171.09	157.23	80.12	80.84	80.64	100.04	109.90	92.24
4.0	92.85	95.63	106.51	54.66	53.02	55.17	79.30	96.34	87.21
2.0	26.32	34.33	37.93	21.60	15.65	18.73	42.75	75.20	63.29
1.0	24.37	27.04	31.77	13.59	14.31	14.11	21.60	37.10	39.77
0.5	8.46	9.79	11.54	7.84	7.54	6.10	14.41	26.53	39.88

A4.3 Total antioxidant capacity

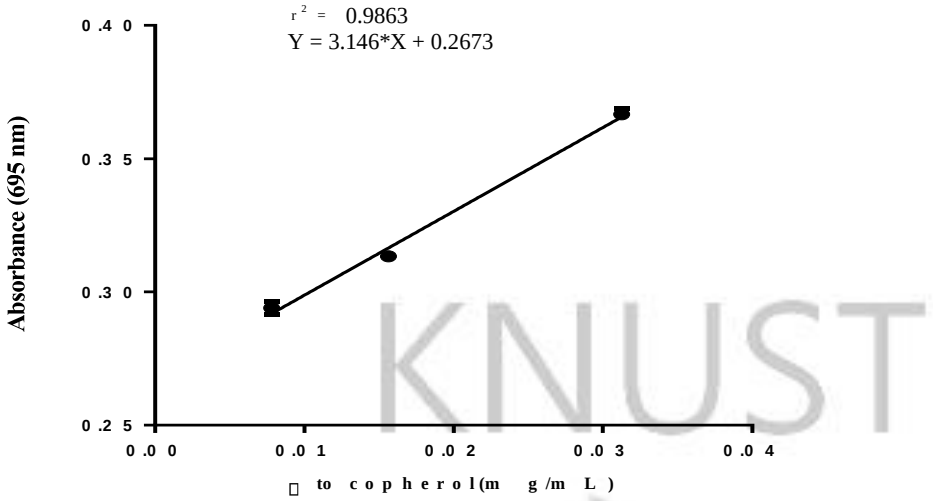

Figure 4.3: α-tocopherol calibration curve for total antioxidant capacity determination

Table 4.3: Total antioxidant capacity of extracts expressed as α-tocopherol equivalence

Conc (mg/mL)	HLML			LOML			HLMR		
10.0	705.24	715.42	676.95	447.46	488.46	501.18	558.39	645.17	662.97
8.0	532.33	509.76	509.44	473.84	450.00	470.34	437.60	545.04	546.63
6.0	488.14	528.19	512.62	427.11	431.88	395.65	431.56	479.56	539.32
4.0	453.18	384.84	385.47	332.07	395.96	380.39	379.12	379.75	422.66
2.0	276.45	326.03	288.21	216.69	258.33	300.29	268.50	321.90	326.99
1.0	243.07	229.08	241.80	192.53	196.03	199.21	248.47	307.60	269.45
0.5	240.21	150.57	220.50	177.59	173.78	179.82	273.59	271.68	251.65

APPENDIX 5: Anti-inflammatory activity

A 5.1 Preparation of Carrageenan suspension

A 1 % w/v carrageenan suspension was prepared by suspending 100 mg of carrageenan powder in 10 mL of 0.9 % w/v saline solution. The suspension was stirred to ensure uniform suspension and allowed to stand on the bench at 25ºC for 23 hours before use.

A 5.2 Prophylactic anti-inflammatory activity of extracts.

Table 5.2.1 Percentage paw eodema inhibition of aspirin, negative control LOML, HLML and HLMR extracts in prophylaxis studies

time/hr	0	1	2	3	4	5	6
Control	0.00 0.00	56.58	62.35	56.79	52.67	46.30	45.88
		57.87	62.58	62.17	56.85	53.58	49.69
	0.00	55.65	57.29	53.18	48.25	43.33	40.04
	0.00	62.76	63.81	60.88	55.02	53.97	52.72
Aspirin 100 mg/mL	0.00 0.00	47.16	41.29	29.55	24.43	16.48	13.07
	0.00	46.12	42.04	40.20	29.39	21.84	16.94
		37.66	32.98	30.21	29.36	25.11	18.94
	0.00	40.29	33.68	29.13	26.86	24.17	23.35
LOML 30 mg/kg	0.00 0.00	50.97	53.98	52.26	45.81	41.72	36.56
	0.00	52.39	55.13	53.53	48.97	46.70	44.19
		53.15	55.75	42.52	47.94	36.01	34.06
	0.00	51.75	56.67	54.00	42.92	38.19	35.11
LOML 100 mg/kg	0.00 0.00	47.69	50.00	46.22	40.97	35.50	31.30
	0.00	43.72	45.14	39.68	35.43	32.79	28.34
		48.89	55.35	41.01	38.38	34.34	29.49
	0.00	49.69	54.09	51.36	39.20	35.01	32.08
LOML 300 mg/kg	0.00 0.00	41.43	41.83	39.04	32.47	27.89	22.71
	0.00	38.63	39.02	36.47	32.94	29.22	22.55
		45.07	48.09	39.03	36.02	31.79	24.95
	0.00	45.97	51.27	49.15	36.86	30.93	29.87
HLML 30 mg/kg	0.00 0.00	40.31	45.49	41.65	29.37	27.64	26.30
	0.00	37.90	48.41	47.84	46.15	40.34	37.90
		35.10	38.71	37.19	31.12	22.39	20.49
	0.00	28.21	34.63	32.68	31.13	28.21	24.51
HLML 100 mg/kg	0.00 0.00	47.00	58.59	55.28	49.69	44.10	42.24
	0.00	43.91	55.51	51.84	47.00	44.10	41.59
		26.12	33.96	31.34	26.12	19.03	17.72
	0.00	55.68	56.80	56.42	55.87	53.82	47.49
HLML 300 mg/kg	0.00 0.00	26.73	41.31	40.00	34.39	25.05	20.75
	0.00	26.98	39.81	34.72	28.68	24.53	23.77
		31.10	42.72	36.81	35.63	32.28	31.10
	0.00	20.84	28.68	27.34	24.67	21.03	17.97
HLMR 30 mg/kg	0.00 0.00	50.38	54.18	52.66	50.57	46.39	45.06
		41.10	52.12	48.94	47.88	43.22	41.53
	0.00	51.59	57.94	52.38	45.63	44.64	41.67
	0.00	50.00	51.91	49.62	48.47	46.37	42.18

HLMR 100 mg/kg	0.00	0.00	48.05	49.13	40.69	37.66	32.90	31.39
	0.00		40.96	47.69	40.96	37.12	32.50	27.50
			45.68	47.16	42.53	37.47	35.16	32.42
	0.00		43.29	49.34	44.05	40.08	34.97	31.57
HLMR 300 mg/kg	0.00	0.00	45.74	47.52	39.41	36.44	30.50	25.54
	0.00		29.50	36.18	34.32	30.43	27.27	25.97
			35.22	39.92	34.46	29.38	22.03	19.40
	0.00		35.96	41.57	36.89	35.77	32.40	26.03

A 5.3 Therapeutic anti-inflammatory activity of extracts.

Table 5.3.1 Percentage paw eodema inhibition of aspirin, negative control LOML, HLML and HLMR extracts in therapeutic studies

time/hr	0	1	2	3	4	5	6		7
Control	0.00	28.91	89.94	103.98	116.51	113.09	110.44		102.09
	0.00	28.91	69.47	88.00	96.00	90.53		88.42	85.89
	0.00	28.91	67.81	91.42	100.00	96.57		94.85	90.99
	0.00	28.91	93.83	101.65		98.77		95.47	91.77
				34.29	108.23				
Aspirin 100 mg/kg	0.00	28.91	33.06	38.12	29.39	27.55	35.33	23.06	13.06
	0.00	28.91	37.33	38.11	37.52	34.18		34.13	27.74
	0.00	28.91	31.63		36.94			29.86	24.56
	0.00	28.91	28.91	38.22	34.46	31.68		29.31	23.17
LOML 300 mg/kg	0.00	28.91	38.80	41.40	37.80	30.80	34.88	26.00	21.60
	0.00	28.91	39.72	45.77	40.93	30.75		30.04	28.43
	0.00	28.91	38.09	42.77	37.88			30.14	23.22
	0.00	28.91	40.08	47.35	36.15	32.02		30.45	25.54
LOML 100 mg/kg	0.00	28.91	47.60	50.52	46.14	41.54	39.02	35.07	27.14
	0.00	28.91	45.49	44.71	42.35	33.70		36.27	30.59
	0.00	28.91	39.93	45.60	40.66			31.68	28.39
	0.00	28.91	45.56	48.52	46.48	34.81		33.33	28.15
LOML 30 mg/kg	0.00	28.91	48.95	52.58	47.42	40.15		36.14	34.61
	0.00	28.91	42.88	45.29	44.18	40.67		35.67	34.01
	0.00	28.91	43.22	44.57	45.16	42.05		39.15	32.95
	0.00	28.91	53.11	49.10	45.29	44.09		37.88	33.27
HLML 300 mg/kg	0.00	28.91	54.45	62.30	61.78	56.20	63.33	47.64	44.33
	0.00	28.91	68.25	74.91	70.00	68.64		57.72	54.04
	0.00	28.91	71.60	74.39	73.34			58.71	56.27
	0.00	28.91	65.93	76.35	73.35	66.73		64.53	63.13

HLML 100 mg/kg	0.00	28.91	45.32	57.30	55.81	51.69 48.89	48.50	45.32
	0.00	28.91	50.93	51.85	55.19	55.77	43.70	35.37
	0.00	28.91	63.08	71.54	62.50		48.08	45.77
	0.00	28.91	60.97	65.47	61.15	58.99	52.16	48.38
HLML 30 mg/kg	0.00	28.91	66.78	72.03	66.44	64.24 75.18	61.02	59.83
	0.00	28.91	69.64	82.14	80.71		74.11	73.04
	0.00	28.91	43.83	51.67	47.67	41.33	38.83	36.83
	0.00	28.91	51.25	61.21	53.02	50.36	48.75	41.64
HLMR 300 mg/kg	0.00	28.91	52.60	56.08	56.77	53.82 42.64	52.26	51.74
	0.00	28.91	48.05	44.50	60.74	64.15	32.99	31.98
	0.00	28.91	48.58	59.13	65.83		61.64	54.10
	0.00	28.91	64.63	73.17	79.44	72.47	70.56	56.27
HLMR 100 mg/kg	0.00	28.91	46.38	51.68	53.44	51.50 83.05	49.03	40.04
	0.00	28.91	77.90	88.76	86.67	69.87	79.81	69.71
	0.00	28.91	71.72	77.95	78.45		62.12	57.24
	0.00	28.91	68.05	72.74	82.33	76.69	74.06	69.74
HLMR 30 mg/kg	0.00	28.91	68.92	76.23	75.50	66.91 60.47	63.44	57.22
	0.00	28.91	60.29	71.20	69.77		57.42	52.24
	0.00	28.91	57.99	65.99	58.36	53.72	51.30	46.84
	0.00	28.91	51.89	55.83	51.17	47.04	42.37	38.42

APPENDIX 6: Wound healing activity A6.1 Preparation of aqueous cream

The aqueous cream used for the wound healing experiment was prepared according to the method described in the British Pharmacopoeia (BP) 2000. The preservative was not included in the preparation to prevent its interference with the antimicrobial activity of the extracts.

Table 6.1.1: Preparation of emulsifying ointment

EMULSIFYING OINTMENT	
Ingredients	Master formula (g)
Emulsifying wax	300
White soft paraffin	500
Liquid paraffin	200
Total	1000

Table 6.1.2: Preparation of aqueous cream BP

AQUEOUS CREAM BP	
Ingredients	Master formula (g)
Emulsifying ointment	300
Phenoxyethanol	10
Sterile distilled water	690

The phenoxyethanol was excluded during the preparation and replaced with distilled water. The weight of water was calculated from its density (1 g/mL).

One hundred grams of aqueous cream was prepared by mixing 30 g emulsifying ointment in 70 mL of sterile distilled water. The mixture was heated on a water bath and stirred in one direction until a homogenous mixture was obtained. The mixture was then allowed to cool. The extracts were incorporated into the homogenous cream to formulate creams of various concentrations of extracts (5 and 10% w/w).

Table 6.1.2: Percentage wound closure of untreated, vehicle, HLML, HLMR and LOML-treated wounds

Days	0	1	3	5	7	9	11	13	15
Untreated	0.00	26	15.79	21.05	26.32	31.58	42.11	47.37	57.89
	0.00	0.00	5.00	10.00	15.00	20.00	30.00	45.00	55.00
	0.00	4.76	9.52	9.52	14.29	19.05	23.81	33.33	42.86
	0.00	5.00	10.00	15.00	20.00	25.00	25.00	35.00	45.00
	0.00	5.26	10.53	15.79	21.05	26.32	31.58	36.84	47.37
Vehicle	0.00	5.88	17.65	23.53	29.41	35.29	41.18	47.06	52.94
	0.00	10.53	21.05	21.05	26.32	31.58	42.11	47.37	57.89
	0.00	5.88	11.76	17.65	23.53	35.29	47.06	52.94	52.94
	0.00	11.11	11.11	27.78	33.33	33.33	44.44	44.44	50.00
	0.00		10.53	15.79	21.05	26.32	36.84	42.11	47.37
SS 1%w/w	0.00	5.26	20.00	32.00	44.00	48.00	56.00	64.00	80.00
	0.00	12.00	16.67	25.00	37.50	41.67	54.17	66.67	83.33
	0.00	12.50	12.50	20.83	29.17	37.50	50.00	66.67	79.17
	0.00	8.33	14.81	25.93	40.74	55.56	62.96	74.07	81.48
	0.00	7.41	22.73	31.82	40.91	54.55	63.64	72.73	81.82
10% HLML	0.00	9.09	21.74	30.43	34.78	39.13	52.17	60.87	78.26
	0.00	8.70	24.00	32.00	40.00	52.00	56.00	68.00	76.00
	0.00	8.00	13.64	27.27	36.36	45.45	54.55	68.18	77.27
	0.00	9.09	19.05	28.57	38.10	47.62	61.90	71.43	80.95
	0.00	9.52							

	0.00	12.00	20.00	28.00	36.00	48.00	60.00	72.00	80.00
5% HLML	0.00	8.33	16.67	29.17	37.50	37.50	50.00	58.33	75.00
	0.00	13.64	22.73	31.82	36.36	36.36	45.45	59.09	77.27
	0.00	14.29	23.81	33.33	42.86	47.62	57.14	66.67	76.19
	0.00	8.00	16.00	28.00	40.00	48.00	56.00	64.00	72.00
	0.00	12.50	20.83	33.33	45.83	54.17	62.50	70.83	75.00
10% HLMR	0.00	17.39	26.09	34.78	47.83	47.83	56.52	65.22	82.61
	0.00	13.64	27.27	31.82	36.36	50.00	59.09	68.18	81.82
	0.00	8.33	20.83	33.33	45.83	54.17	62.50	70.83	79.17
	0.00	13.04	21.74	30.43	39.13	56.52	65.22	73.91	82.61
	0.00		16.00	24.00	36.00	48.00	56.00	64.00	80.00
5% HLMR	0.00	8.00	24.00	32.00	44.00	48.00	52.00	64.00	76.00
	0.00	12.00	26.09	34.78	43.48	47.83	56.52	65.22	78.26
	0.00	17.39	18.18	27.27	40.91	50.00	59.09	68.18	77.27
	0.00	9.09	20.00	28.00	40.00	48.00	56.00	64.00	72.00
	0.00	8.00 8.70	17.39	26.09	30.43	39.13	47.83	56.52	73.91
10% LOML	0.00	8.00 12.50	20.00	32.00	44.00	48.00	52.00	64.00	84.00
	0.00	13.04	25.00	37.50	45.83	50.00	58.33	70.83	79.17
	0.00		26.09	39.13	47.83	56.52	65.22	73.91	82.61
	0.00	8.00	16.00	28.00	40.00	56.00	64.00	72.00	80.00
	0.00	0 4.35	17.39	30.43	39.13	52.17	65.22	73.91	82.61
5% LOML	0.00	8.7	17.39	26.09	34.78	34.78	52.17	60.87	78.26
	0.00	8.33	16.67	29.17	41.67	45.83	58.33	66.67	75.00
	0.00	9.09	18.18	27.27	31.82	36.36	45.45	54.55	72.73
	0.00	9.52	19.05	28.57	38.10	47.62	57.14	66.67	76.19
	0.00	12.50	20.83	25.00	33.33	41.67	50.00	62.50	70.83