EVALUATION OF THE PROPERTIES OF *CROTON LOBATUS* LINN. (EUPHORBIACEAE) AND *SANSEVIERIA LONGIFLORA* SIMS (AGAVACEAE) LEAF EXTRACTS ON HEALING OF SURGICALLY CREATED CUTANEOUS WOUNDS IN RATS

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DEPARTMENT OF VETERINARY SURGERY AND RADIOLOGY,
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Declaration

I declare that the work in this dissertation entitled "**Evaluation of the properties of *Croton lobatus* linn. (euphorbiaceae) and *Sansevieria longiflora* sims (agavaceae) leaf extracts on healing of surgically created cutaneous wounds in rats**" has been performed by me in the Department of Veterinary Surgery and Radiology, Ahmadu Bello University, Zaria, under the supervision of Prof. A. Z. Hassan and Dr S. T. Fadason. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

MUHYIDEEN ADIO KILANI ____________________

Name of Student Signature

Certification

This dissertation entitled "**EVALUATION OF THE PROPERTIES OF *CROTON LOBATUS* LINN. (EUPHORBIACEAE) AND *SANSEVIERIA LONGIFLORA* SIMS (AGAVACEAE) LEAF EXTRACTS ON HEALING OF SURGICALLY CREATED CUTANEOUS WOUNDS IN RATS**" by Muhyideen Adio KILANI, meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Prof. A. Z. Hassan Chairman Supervisory committee	____________________ Signature	________________ Date
Dr. S. T. Fadason Member, Supervisory committee	____________________ Signature	________________ Date
Dr. S. T. Fadason Head of Department	____________________ Signature	________________ Date
Prof. S. Z. Abubakar Dean, School of Postgraduate Studies, A.B.U. Zaria	____________________ Signature	________________ Date

Dedication

This work is dedicated to my beloved mother (Alhaja Fatimat Kilani Ola Iya) and grandmother (Alhaja Hawanat Abeke Agoro). May Almighty Allah forgive them, bless them and make paradise their final abode.

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Abstract

This study was carried out to evaluate the dermal wound healing potential and analgesic properties of water extract of these plants using excision wound model in white rats. Twenty six white rats (male and female) of 22 weeks old, weighing 180 to 200g were used for the assessment of wound healing. For the evaluation of wound healing properties of *C. lobatus*, 13 animals were divided in to 4 groups. Group I-III had 3 animals each that were used for the estimation of percentage wound contraction, epithelialisation time, microbial culture and tensometry while the group IV, containing 4 animals were used for histology and biochemical tests. This was repeated for *S. longiflora* using another set of 13 white rats. Full thickness skin excision of 1.0 X 1.0 cm was surgically created on 4 different part of the dorsum of each rat under anaesthetic condition using ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg). Two wounds were treated using the local methods of preparations and water extracts respectively, the 3rd was treated with normal saline while the 4th wound was treated with Cicatrin powder as positive control. One animal was sacrificed from each of the *C. lobatus* and *S. longiflora* groups (IV and VIII) on the day 4, 7, 10 and 13 (post wounding) for histopathological evaluation while granulation tissue was harvested for the estimation of hydroxyproline, hexosamine, uronic acid and total protein content. Agar diffusion method was used to determine the antimicrobial properties of varied concentration of the *C. lobatus* and *S. longiflora* against culture of *Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*. Incisional wound model was used to test for analgesic properties of *C. lobatus* and *S. longiflora.* Eight white rats were divided into 2 groups. Four incisions of 1cm long were surgically created on 4 different part of the dorsum of each rat.

C. lobatus water extracts was applied on the 1st group while the *S. longiflora* water extracts was applied on the 2nd group. Normal saline was used as negative control while xylocaine cream was used as positive control. Algometer was used to measure the pain perception over a period of 5 days. The ease of wound debridement in *C. lobatus* and *S. longiflora* treated wounds were significantly ($p<0.0001$) higher than that of Cicatrin treated wounds. Wound contraction in *C. lobatus* and *S. longiflora* treated wounds were significantly higher ($p<0.001$) than the Cicatrin treated wounds. Wound epithelialisation in *C. lobatus* and *S. longiflora* treated wounds were significantly higher ($p<0.001$ and $p<0.05$ respectively) than the Cicatrin treated wounds. Histopathology studies revealed that *C. lobatus* and *S. longiflora* plant extracts promoted formation of healthy granulation tissues, fewer inflammatory cell infiltration, fibroblast proliferation and collagen synthesis. The period of epithelialisation of the wounds treated with water extract of *C. lobatus* and *S. longiflora,* normal saline and Cicatrin powder were 11.22 ± 0.40, 12.5 ± 0.65, 13.75 ± 0.45 and 17.56 ± 1.39 days respectively. The faster rate of wound healing in *C. lobatus* and *S. longiflora* water extract was supported by the increased hexosamine content. There was no significant difference between the mean tensile strength values of all the treated wounds (plant extracts, normal saline and Cicatrin) at day 22 after injury. *Croton lobatus* and *S. longiflora* water extract inhibited the growth of the common wound microbial organisms. The water extracts of these plants also possessed the ability to increase pain threshold in healing wounds. It was concluded that water extracts of *C. lobatus* and *S. longiflora* significantly promoted wound healing; hence in the future, they can serve as better agents for wound management though, *C. lobatus* showed a better activity.

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Lists of Abbreviation

Croton lobatus (*C. lobatus*)

Sansevieria longiflora (*S. longiflora*)

A (wound treatment with *Croton lobatus* paste)

B (wound treatment with *Croton lobatus* water extract)

C (wound treatment with Normal saline)

D (wound treatment with Cicatrin powder)

E (wound treatment with *Sansevieria longiflora* sap)

F (wound treatment with *Sansevieria longiflora* water extract)

G (wound treatment with xylocaine cream)

CFU: colony forming units

CFC: colony forming counts

MSA: mannitol sugar agar

EMB: eosin methylene blue agar

SD: sabouraud dextrose agar

NA: nutrient agar

MAC: Mac Conkey agar

SSA: Salmonell-shigella agar

LF: Coliform

N.LF: Non-coliform

CL-P: *Croton lobatus* paste

CL-0.5: *Croton lobatus* (0.5 mg/ml)

CL-0.05: *Croton lobatus* (0.05 mg/ml)

CL-0.005: *Croton lobatus* (0.005 mg/ml)

CRO 30: Ceftriaxone (30 µg)

E 15: Erythromycin (15 µg)

STX 25: Sulphamethoxazole (25 µg)

OB 5: Cloxacillin (5 µg)

OX 1: Oxacillin (1 µg)

CAZ 10: Ceftazidime (10 µg)

CN 10: Gentamycin (10 µg

AML 25: Amoxycillin (25 µg)

SL-P: *Sanseviera longiflora* paste.

SL-0.5: *Sanseviera longiflora* (0.5 mg/ml)

SL-0.05: *Sanseviera longiflora* (0.05 mg/ml)

SL-0.005: *Sanseviera longiflora* (0.005 mg/ml).

WHO: World Health Organization

Millimeter (mm)

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

A wound has been defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue (Kokane *et al.*, 2009). The prevalence of chronic wounds in the human community was reported as 4.5 per 1000 population while acute wounds was more than 10.5 per 1000 population (Kumar *et al.*, 2007). Wound is commonly encountered in clinical practice. Wound healing on the other hand refers to the body's natural process of restoration of tissue architecture and function after an injury (Reddy *et al.*, 2008). It involves inflammation, cell proliferation, and contraction of collagen lattice formation (Reddy *et al.*, 2008). Wounds can be broadly categorized into acute or chronic forms while on etiological base, it includes surgical wounds, traumatic wounds, lacerations and burns (Kokane *et al.*, 2009).

Wound healing process is characterized by increased hydroxyproline levels in the granulation tissue, hence measurement of the hydroxyproline could be used as an index for collagen turnover (Woessner, 1961). By correlating hexosamine content of wounds, it can be judged how fast the wound heals. It is important to note that hexosamine content will increase during wound healing process and decreases when maturation and remodeling phase is attained (Rondle and Morgan, 1955). Chitra *et al*. (1998) found that an increase in uronic acid level in treated animals represents an enhanced synthesis of glycosaminoglycans.

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind (Sheela *et al.*, 2012). Plants have been used for the treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns from the ancient time (Kumar *et al.*, 2007).

Croton lobatus L. (*Euphorbiaceae*), a tropical wild plant is commonly used in African traditional medicine to prepare remedies for many diseases (malaria, pregnancy troubles, dysentery) (Aké, 1975). Weniger *et al*. (2004) reported the *in vitro* antiplasmodial activity of the aerial parts and roots crude extracts of *Croton lobatus*. A leaf-paste of *Croton lobatus* is rubbed on to guinea-worm sores to aid healing (Burkill, 1985). Heated leaves are rubbed on to areas of costal and rheumatic pain, and a leaf-decoction by mouth, or a bark-decoction by enema is given as a purgative (Burkill, 1985). It is used as pain-killer, laxative, treat cutaneous and subcutaneous parasitic infection, menstrual cycle disorders, as antidotes (venomous stings and bites), treatment for paralysis, epilepsy, convulsions, spasm and topically to treat ulcers, sores, and headache (Burkill, 1985). Odukoya *et al.* (2012) reported that *Croton lobatus* Linn. (*Euphorbiaceae*) is usually boiled with water and the decoction is then taken to treat wounds that refuse to heal while Bouquet and Debray (1974) reported the whole plant infusion to be used as a topical bath to treat skin diseases.

The medicinal uses of *Sansevieria* species include treatment of abdominal pains, ear ache, diarrhea and hemorrhoids (Van Wyk *et al*., 1997; Aliero *et al*., 2008). Traditionally, in treating ear aches and hemorrhoids, the leaves are heated and the warm juice is squeezed onto the affected area. The leaf sap is applied directly to infected sores, cuts and grazes. It is also used to treat fungal and scabies infection (Traditional Medicine Database, 2002;

Olivia, 2005). Previous studies on *Sansevieria* species have documented anti inflammatory activity (Mortan, 1981; Da Silva *et al*., 2003) analgesic property (Sunilson *et al*., 2009), antioxidant and antimicrobial activity (Onah *et al*., 1994; Aliero *et al*., 2008). In Nigeria, the leaves and roots of *Sansevieria liberica* are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhoea, eczema, gonorrhea, hemorrhoids, hypertension, menorrhagia, piles, sexual weakness, snake bites and wounds of the foot (Gill, 1992; Osabohien and Egboh, 2008; Adeyemi *et al*., 2009). The sedative and anticonvulsive activities of the roots have been reported by Adeyemi *et al*. (2007). Recent studies on *S. roxburghiana* rhizome demonstrated remarkable anti-diabetic activity in streptozotocin induced diabetic rats (Pallab *et al*., 2010) and the leaf sap is applied directly to infected sores, cuts and grazes (Sardessai *et al*., 2014). Anbu *et al*. (2009) reported the analgesic effects of ethanol and water extracts of *Sansevieria trifasciata* leaves while the ethanol extract significantly reversed yeast-induced fever.

Recent interview with the local people in the South Western part of Nigeria revealed that *Croton lobatus* is used to treat wounds by applying its fresh leaves or dried powder on wound surface while the leaf-decoction is used to aid development of fetus in the womb. Similarly the leaves of *Sansevieria longiflora* were heated and the warm sap was squeezed onto the wound surface. This is traditionally used to manage deep wounds and chronic wounds that failed to heal. The root decoction has been suggested to be useful in the treatment of whooping cough and diabetes in human.

1.2 Statement of Research Problems

It has been estimated that 80% of the population of developing countries is unable to afford pharmaceutical drugs and rely on traditional herbal medicines to sustain their primary health care needs (Survase and Raut, 2011). The prevalence of wounds coupled with the poor economic condition of several countries necessitates an evaluation of affordable options for managing wounds. To the best of our knowledge no controlled study has been conducted to validate the traditional use of *Croton lobatus* and *Sansevieria longiflora* in the management of wounds.

1.3 Justification of the Research

The World Health Organization estimates that around 80% of the world population in developing countries rely on traditional plant medicines for primary healthcare needs, of which a major proportion corresponds to plant extracts or their active principles (Sampson *et al*., 2000; WHO, 2008). Reasons for the use of traditional medicines may vary, beside the fact that the plant preparations are relatively cheaper, adverse drug reactions are rarely observed when compared with synthetically produced pharmaceuticals (Chariandy *et al*., 1999). Various studies have established that herbal medicines are safe, effective and less costly alternatives to synthetic medicines that treat certain bacterial infections (Vermani and Garg, 2002).

Due to the profitable efficiency of medicinal plants on biological activities, there is a need for validation of the believes in traditional use of *Croton lobatus* and *Sansevieria longiflora* in the management of wounds and future isolation of newer biological compounds from plants which can serve as novel therapeutic agents.

1.4 Aim of the Study

The present study was undertaken to fully evaluate the dermal wound healing potential and analgesic properties of *Croton lobatus* and *Sansevieria longiflora* extracts on surgically created cutaneous wounds in rats serving as model for higher animals.

1.5 Aim and Objectives

1.5.1 Specific aim:

The study was conducted to purely validate or otherwise the traditional medical practice of applying *Croton lobatus* and *Sansevieria longiflora* leaf paste and sap respectively for wound management.

1.5.2 Objectives:

- To study different stages of wound healing and evaluate the effects of *Croton lobatus* and *Sansevieria longiflora* water extracts on wounds in rats.
- To study the biochemical features of wounds treated with *Croton lobatus* and *Sansevieria longiflora* water extracts in rats.
- To evaluate the anti microbial properties of *Croton lobatus* and *Sansevieria longiflora* water extracts *in vitro*.
- To evaluate the analgesic properties of *Croton lobatus* and *Sansevieria longiflora* water extracts on incisional wounds in rats.

1.6 Research Questions

- Do *Croton lobatus* and *Sansevieria longiflora* paste and water extracts aid in wound healing as traditionally reported?

- Is there any effect of the topical use of these plants on biochemical parameters of the wounds?
- Do *Croton lobatus* and *Sansevieria longiflora* paste and water extracts have antimicrobial properties?
- Do *Croton lobatus* and *Sansevieria longiflora* paste and water extracts have analgesic properties?

CHAPTER TWO

LITERATURE REVIEW

2.1 *Croton lobatus* Linn.

Croton is an extensive plant genus of the family Euphorbiaceae established by Carolus Linnaeus in 1737 (Anonymous 1). The flowering plant family Euphorbiaceae, includes 313 genera and over 8100 species that are cosmopolitan in distribution (Anonymous 1). Croton is a “giant genus,” with 1223 species accepted in The World Checklist and Bibliography of Euphorbiaceae, but others put the number of species under Croton at 1797 starting with *Croton abaitensis* (1st species) and ending in *Croton zeylanicus* (1797th species) (Anonymous 2). The genus name comes from the Greek word “Kroton”, which means ticks, because of the seeds resemblance to ticks (Anonymous 1). All the species under Croton are herbs, shrubs, trees and occasionally lianas (climbers) that are ecologically prominent and important elements of secondary vegetation in the tropics and subtropics worldwide (Anonymous 1).

2.1.1 Taxonomic serial number

28281 (Malpighiales of North America Update database version 2011)

2.1.2 Common name

Rusffoil; Croton; Lobed croton (English) (Malpighiales of North America Update database version 2011). In Nigeria, the plant is known in the three major languages as gaásàyaá (*Hausa)*; òkwè-one a kind of bean (*Igbo)*; àjẹ́ofòlé or ẹ̀ru (*Yoruba)* (Burkill, 1985).

Malpighiales of North America Update database version, 2011 also reported the plant to be known as Astraea *lobata* (L.) Klotzsch. *Croton lobatus* is shown in the Plate 2.1a in page 8.

Plate 2.1a: The picture of the *Croton lobatus* used in the management of wounds in Nigeria.

Plate 2.1b: The picture of the fruits of *Croton lobatus*

2.1.3 Taxonomy hierarchy

Catalogue of Life, (2016) has classified *Croton lobatus* as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Euphorbiales

Family: Euphorbiaceae

Subfamily: Crotonoideae

Genus: Croton

Species: *lobatus*

Scientific name: *C. lobatus*

The GBIF backbone Taxonomy, (2016) has also classified *Croton lobatus* as follows:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Genus: Astraea

Species: *Astraea lobata*

2.1.4 Origin and distribution

C. lobatus L. is native of Caribbean, North American, South America and West Indies, and was introduced in Senegal, Eritrea, Ethiopia, Arabian Peninsula and Bangladesh (Malpighiales of North America Update database version 2011; Gaikwad *et al.,* 2012). The genus Croton has about 1300 species of trees, shrubs and herbs distributed in the tropical and subtropical regions of North and South Hemispheres (Block *et al.,* 2004). A number of the Croton species are known for their medicinal qualities especially in Africa, Asia and South America (Yibralign, 2007).

2.1.5 Botanical description

According to Gaikwad *et al*. (2012), *Astraea lobata* (L.) Klotzsch which is recently known as *Croton lobatus* (L.) is an annual plant (monoecious) that grows up tp 60 – 70 cm high with branches and stems ribbed. It is stellate-hairy when young with alternate leaves that is sometimes opposite towards the apex, and is 2.5 – 10 cm long and deeply lobed in 3 – 5 places. The lobes are oblanceolate to obviate with crenate-serrate at the margins, acuminate at apex and sparsely stellate-hairy to almost glabrous. Its petioles are 6 – 15 cm long; stipules, filiform, and small. The plant has a strong tap-root and laterals and it is not easy to root out in cultivated land (Burkill, 1985).

2.1.5.1 Flowering and fruiting: Gaikwad *et al.*, (2012) reported that *Croton lobatus* usually fruits between the month of June and September (Gaikwad *et al.*, 2012). It inflorescences a 12 cm long raceme that is axillary or terminal, slender with the male and female flowers intermixed on inflorescences or sometimes male flowers in upper half and female flowers in lower half of the plant (Gaikwad *et al.*, 2012). Its flowers are unisexual, with 5 merous that is regular and yellowish green (Gaikwad *et al.*, 2012). The fruit of *Croton lobatus* is shown in the Plate 2.1b above.

2.1.5.2 Male flowers and female flowers: Gaikwad *et al*. (2012) further described the male flower to be buds globose; with slender pedicels that is 1 – 2 mm long; Sepals elliptic that is 1 mm long and obtuse at the apex. The petals are slightly shorter than sepals, obviate, and obtuse at the apex. The stamens which are about 10 – 13 are apically inflexed in bud. The filaments are 1 – 2 mm long while the anthers are 0.5 mm long and 2-celled. He further described the female flower to have stout pedicels that is 1 – 2 mm long with persistent Calyx. The lobes are linear-lanceolate, 7 mm long, dentate with minute-hairy at margins. The Ovary is glabrous or sparsely stellate-hairy. The styles are 2 – 4 mm long and each one divided into 2 – 4 linear branches. The capsules are ovoid-oblong, 7 mm in diameter, glabrous or sparsely stellate-hairy and 3-lobed. The seeds are ellipsoid, 6 ×3 mm, truncate at base, prominently carcunculate at apex, verrucose, ash-coloured with brown spots. The carcuncles are conical and yellowish.

2.1.5.3 Habitat: Croton lobatus grows on roadsides and in wastelands (Gaikwad *et al.*, 2012). It is a Neotropics, Africa, Arabia, and a new record for Dominica, on roadside at 15 m, Baiac road (Whitefoord 4622), Cabrit Swamp (Whitefoord 4082), Layout River Valley (Ernst 1132) (Nicolson *et al.*, 19991).

2.1.6 Phytochemical properties

In 2013, Nath *et al*. reported that phytochemicals in the genus croton are considerably diverse, and studies have revealed the presence of sesquiterpenes (Aguilar-Guadarrama and Rios, 2004; Wang and Zou, 2008), diterpenes (McChesney *et al.*, 1991; Sutthivaiyakit *et al.*, 2001), triterpenes (Barbosa *et al.*, 2003), steroids, flavonoids (Cai *et al.*, 1991), and alkaloids (Amaral and Barnes, 1998; Aboagye *et al.*, 2000; Milanowski *et al.*, 2002; Yibralign, 2007). The diterpenoids found in the genus croton include phorbol esters, clerodane, labdane, kaurane, trachylobane, pimarane, cembranoid (Block *et al.*, 2004), neoclerodane, halimane, isopimarane, and secokaurane (Nath *et al.*, 2013), triterpenoids, either pentacyclic or steroidal (Nath *et al.*, 2013), volatile oils containing mono and sesquiterpenoids (Nath *et al.*, 2013), different classes of alkaloids (Nath *et al.*, 2013), phenolic substances like flavonoids, lignoids and proanthocyanidins predominate (Salatino *et al.*, 2007), and volatile oil including eugenol, vanillin, crotsparinine, crotoflorine, oblongi-foliol, triterpenic acid, sparsiflorine, dotricontamol, b-amyrin and b-sitosterol (Anonymous 2). Several species of the genus are aromatic, indicating the presence of volatile oil constituents (Oliveira *et al.,* 2001; Lopes *et al.,* 2003). Like most Euphorbiaceae, *Croton* species contain latex, which is red-colored in some species, a characteristic usually associated with medicinal properties (Sandoval *et al.,* 2002; Risco *et al.,* 2003).

Different parts of Croton species plants have their typical compounds; saponins and resins are usually found in the seeds (PROTA 2011); crotepoxide and crotomacrine in the fruits (Tane *et al*. 2004); stem, barks and twigs contain fatty acids, lupeol, betulin (PROTA 2011); while the roots contain chalcone and secondary metabolites such as 3β-acetoxy tetraxer-14-en-28-oic acid, trachyloban-19-oic acid, trachyloban-18-oic acid, neoclerodan-5,10-en-19,6β; 20,12-diolide, 3α,19-dihydroxy trachylobane, 3α,18,19-trihydroxy trachylobane (Kapingu *et al*. 2000).

The principal ingredient in *C. lobatus* is crotonic acid, probably in combination with toxic albuminoids, and this is found throughout the plant (Kerharo and Bouquet, 1950; Bouquet and Debray, 1974). Brazilian samples were reported to contain tertiary and quaternary alkaloids and haemolytic saponins (Farnsworth, 1969; Willaman and Li, 1970). Burkill, (1985) reported that the plant contain glycosides, saponins, steroids and alkaloids. Recently, several compounds were isolated from the leaves, stem and bark of *C. lobatus* including sterols, diterpenes, triterpenes, polyphenol and one nitrogen compound (Attioua, 2005; Chabert *et al.*, 2006), while sesquiterpene called vomifoliol has been isolated from the leaves and stems (Stuart and Woo-ming, 1975). Although studies on *C. lobatus*, clearly identified one alkaloid (Attioua, 2005; Chabert *et al.,* 2006), Barthelemy *et al.,* (2012) identified five types of alkaloid in *C. lobatus* aerial parts including Palmitanoide, Onosmin B, N-(2-hydroxy-1-phenylpropyl) benzamide, Onosmin A and Aurentiamide acetate.

2.1.7 Wound healing properties

In 1985, Burkill reported that *C. lobatus* is used topically to treat ulcers, sores and headache. The whole *C. lobatus* plant infusion is used as a topical bath to treat skin diseases while hot water infusion of it is drunk by women in the Ivory Coast to induce sterility (Bouquet and Debray, 1974). An ethno medicinal red tree sap called *Sangre de grado* is obtained from *Croton palanostigma*, which is used to treat gastrointestinal ulcers, cancer and to promote wound healing (Sandoval *et al.,* 2002). Odukoya *et al.*, (2012) reported that *C. lobatus* Linn. is usually boiled with water and the decoction is taken to treat wounds that refuse to heal. He also reported that it was among the documented wound healing medicinal plants in which their ethanolic extracts were investigated for free radical scavenging activities and lipid peroxidation.

2.1.8 Antimicrobial properties

Some species of Croton are known to contain proanthocyanidins or alkaloids that may be in form of taspine or some of the several benzylisoquinoline-like compounds (Dadson *et al.*, 2012). Diterpenes, a common compound in croton may be represented as clerodanes, cembranoid, halimanes, kauranes, labdanes, phorbol esters, trachylobanes and sarcapetalanes (Dadson *et al.,* 2012) and volatile oils present in some other Croton species (Salatino *et al*., 2007) have been shown to have antibacterial properties (Peres *et al*., 1997, Abo *et al*., 1999). Biologically active compounds such as sonderianin, korberin A and B isolated from the genus Croton with specific antibacterial activities against *Mycobacterium* *smegmatis, Staphylococcus aureus, Bacillus subtilis,* and *E. coli* are also well known (McChesney *et al*. 1991).

Dadson *et al.,* (2012) found that organic extracts of Croton species inhibit both Gram positive and Gram negative bacteria while the inorganic extracts were ineffective but acetone extracts inhibited the growth of the antibiotic sensitive strain of *M. tuberculosis*. He further showed that the extracts of Croton species could be used as alternative therapy in treatment of bacterial infections or used as adjuvant agents in antibiotic therapy against pathogenic bacterial infections

2.1.9 Anti inflammatory and analgesic properties

Croton lobatus is used generally as healing leaf medicines, pain-killers, laxatives, cutaneous and subcutaneous parasitic infection, menstrual cycle disorders, antidotes (venomous stings and bites), paralysis, epilepsy, convulsions and spasms (Burkill, 1985). A decoction is reported as a children’s medicine (Dalziel, 1937) but the purpose is not indicated. In Senegal the leaves are used with another undetermined plant for whooping-cough, all spasmodic coughing, and for stomatitis (Kerharo and Adam, 1974). In the Gambia there is unspecified medicinal use (Fox 134, unpublished) while in Nigeria it is used to assuage the pain of scorpion-sting (Daggash, unpublished).

2.1.10 Antioxidants properties

Literature survey revealed that the essential oils from northeastern Brazilian Croton species, *Croton zenthmeri*, *Croton nepetaefolius* and *Croton argyrophylloides* exhibited good antioxidant activities (Morais *et al.,* 2006). The crude essential oil obtained from the stem bark of *Croton urucurana* exhibited antioxidant properties with the main components of the antioxidant fraction being α-bisabolol, α-eudesmol and guaiol (Simionatto *et al.*, 2007). Nath *et al.*, (2013) have also reported the antioxidant properties of *Croton lechleri*, *Croton cajucara*, *Croton argyratus*, and *Croton caudatus.*

2.1.11 Antineoplastic properties

Recently the use of the powdered roots of *Croton roxburghii* Balakr (known as Hongkai in Arunachal Pradesh), in the treatment of cancer by the Kati tribe of Arunachal Pradesh have been briefly reported (Anonymous 2). Also the use of *Croton caudatus* Geisel in the treatment of cancer in the Saikot area of Manipur has been recently reported (Anonymous 2). Nath *et al*. (2013) reported the anticancer properties of *Croton tiglium* L, *Croton cajucara* (Sacaca), *Croton zambesicus*, *Croton tonkinensis*, *Croton oblongifolius*, *Croton eluteria*, *Croton brasiliensis*, *Croton membranaceus*, *Croton malambo*, *Croton flavens*, *Croton palanostigma*, *Croton pierrei*, *Croton regelianus*, *Croton matourensis*, *Croton tiglium*, *Croton argyratus*, *Croton barorum* and *Croton goudotii*.

2.1.12 Traditional or medicinal use

Although *Astraea lobata* is reported to be an alternative host of nematodes (Meloidogyne spp.), fungi causing powdery mildew, and of cucumber mosaic virus (Schmelzer, 2007) and also reported that there is a threat of introduction of plant diseases along with this species in India (Gaikwad *et al.,* 2012), yet it is reported to be used traditionally to manage different ailments. In Ivory Coast arrow-poison made from *Croton lobatus* plant is reputed to be the most poisonous used, the plant is simply crushed between stones with a little water and the arrows dipped in the paste (Burkill, 1985). The leaves boiled in a little water are used as an enema for gynaecological affections, and leaf-paste is rubbed on to guinea-worm sores (Burkill, 1985). Heated leaves are rubbed on to areas of costal and rheumatic pain, and a leaf-decoction by mouth, or a bark-decoction by enema is given as a purgative (Burkill, 1985). Weniger *et al.*, (2004) have reported the in vitro antiplasmodial activity of its aerial parts and roots crude extracts. Aké (1975) also reported *C. lobatus* L. be used in African traditional medicine to prepare remedies for many diseases like malaria, pregnancy troubles and dysentery.

Burkill, (1985) reported that the plant is used for religious, superstitious, and magic purposes. In Nigeria the plant has superstitious use against witchcraft, the person bath with a lotion prepared from it to keep evils at bay (Dalziel, 1937).

The *Croton bonplandianus* Bail., *Croton caudatus* Geisel., *Croton chlorocalyx* Linn., *Croton joufra Roxb*., *Croton roxburghii* Balakr and *Croton tiglium* Linn. are used in the treatment of various diseases, disorders and ailments like antifertility, boils, bowel complaints, chicken pox, cholera, cold and coughs, constipation, cuts and wounds, diarrhoea, dysentry, eye diseases, epilepsy, fever, gastric disorders, insanity, jaundice, liver complaints, malaria, rheumatism, ringworms, scurvy, spasmolytic agent, snake bite, and sprains (Anonymous 2). Other species are used in the treatment of cancer, constipation, diabetes, digestive problem, dysentery, external wounds, intestinal worm, pain, ulcers and weight loss (Salatino *et al*., 2007), fevers, constipation, diarrhea, intestinal worms, malaria, pain ulcers, inflammation (Yibralign, 2007). Table 2.1 was prepared from different literatures consulted on traditional uses of *Croton lobatus.*

Table 2.1. Some Croton species and their traditional uses in various part of the world as derived from different literature.

Species	Region	Plant parts	Traditional uses	Reference
C. cajucara	A	leaves and stem bark	treatment of high blood cholesterol levels and gastrointestinal disturbances, as well as hepatic disturbances and weight loss	(Campos *et al.*, 2002; Grassi-Kassisse *et al.*, 2003*).*
C. celtidifoliu	A	bark and leaf infusions	treatment of inflammatory diseases, leukemia, ulcer and rheumatism	(Nardi *et al.*, 2003).
C. eluteria	A	Its bitter bark	to treat bronchitis, diarrhea and dysentery, fever and malaria	(Vigor *et al.*, 2001).
C. lechleri L	A	Blood red sap	assumed ability to speed up the healing of wounds, purgative and tonic, to treat wounds, as a haemostatic, to accelerate healing and to protect lesions from infection	(Cai *et al.*, 1991; Cai *et al.*, 1993a; Cai *et al.*, 1993b; Milanowski *et al.*, 2002)
C. malambo	A	bark infusion	treatment of diabetes, diarrhea, rheumatism, gastric ulcer and as anti-inflammatory and analgesic	(Suárez *et al.*, 2003)
C. nepetaefolius	A	Infusions or decoctions of the bark and leaves	used as a stomachic, carminative and for the treatment of intestinal colic, antispasmodic properties and to relieve flatulence and to increase appetite	(Moura *et al.*, 1990; Lahlou *et al.*, 2000)
C. palanostigma	A	red latex from the bark	used as wound-healer, treating gastric ulcer and intestinal inflammation	(Porrasreyes *et al.*, 1993; Miller *et al.*, 2000).
C. schiedeanus	A		for the treatment of hypertension	(Guerrero *et al.*, 2001; Guerrero *et al.*, 2002).
C. urucurana	A	Bark	analgesic and anti-inflammatory effects, to treat wound infection, accelerate wound-healing, treat rheumatism, cancer and other illnesses	(Peres *et al.*, 1997; Peres *et al.*, 1998; Orlandi-Mattos *et al.*, 2002).
C. zehntneri	A	bark and leaves	mainly as sedative, appetite stimulating, antianorexigen and for the relief of gastrointestinal disturbances; as sweeteners in foods and drinks	(Coelho-de-Souza *et al.*, 1997; Coelho-de-Souza *et al.*, 1998).
C. arboreous	B		used as an auxiliary anti-inflammatory in respiratory ailments	(Aguilar-Guadarrama and Rios, 2004).

Species	Region	Plant parts	Traditional uses	Reference
C. californicus	B	Leaves	pain reliever for rheumatism	(Chavez *et al.*, 1982)
C. draco	B	Sap	internal use for cough, flu, diarrhea and stomach ulcers, and topically as wound healing for cuts, open sores, herpes, anti-septic after tooth extraction and for oral sores	(Murillo *et al.*, 2001)
C. macrostachys	C	Root, seed	Antidiabetic and purgative	(Mazzanti *et al.*, 1987; Kapingu *et al.*, 2000)
C. zambesicus	C	Leave	to treat fever, dysentery and convulsions, anti-hypertensive, anti-microbial (urinary infections) and to treat malaria-linked fever	(Block *et al.*, 2002; Ngadjui *et al.*, 2002; Block *et al.*, 2004)
C. kongensis	D		Dysmenorrhoea	(Thongtan *et al.*, 2003).
C. oblongifolius	D	Leaves, flowers, fruits, seed, bark, roots	are used as tonic, against flat worms, to treat dysmenorrhoea, as purgative, to treat dyspepsia, to treat dysentery, to treat chronic enlargement of the liver and remittent fever, applied externally to the hepatic region in chronic hepatitis, and to treat gastric ulcers and gastric cancers	(Ahmed *et al.*, 2002; Ngamrojnavanich *et al.*, 2003; Sommit *et al.*, 2003)
C. roxburghii	D	Various parts	against snake poisoning and to treat infertility, fever and wounds	(Singh *et al.*, 1999).
C. sublyratus	D		anti-helminthic and to treat dermatological problems.	(Tansakul and De-Eknamkul, 1998; Gupta *et al.*, 2004).
C. tiglium L	D	Seed	as laxative, tumors and cancerous sores, source of a commercially available oil (croton oil), used as purgative	(Tsai *et al.*, 2004).
C. tonkinensis	D	Leaves	for stomach-ache to treat burns, abscesses, impetigo, dyspepsia and gastric/duodenal ulcers. They are a component of recipes used to treat urticaria, leprosy and psoriasis	(Giang *et al.*, 2003)

KEY: A: South America; B: North America and Central America; C: Africa; D: Asia

2.2 *Sansevieria longiflora* Sims

*Sansevieria*ns are popular garden or indoor plants with long rhizomes and fibrous roots having ability to flourish under low light conditions and requiring minimal attention (Olivia, 2005). In 2008, USDA reported many species of *Sansevieria* to be grown as ornamental plants including *Sansevieria cylindrica, Sansevieria ehrenbergii, Sansevieria guineensis, Sansevieria longiflora, Sansevieria roxburghiana, Sansevieria trifasciata* and *Sansevieria zeylanica*.

2.2.1 Taxonomic serial number

43013

2.2.2 Common name

Florida bowstring hemp, Mother-in-law tongue, bowstring hemp. In Nigeria it is known as Oja'kooko (Yoruba) and Moda (Hausa).

2.2.3 Taxonomic hierarchy

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Sub kingdom	Viridiplantae
Infra kingdom	Streptophyta – land plants
Super division	Embryophyta
Division	Tracheophyta – vascular plants, tracheophytes
Subdivision	Spermatophytina – spermatophytes, seed plants, phanérogames
Class	Magnoliopsida
Superorder	Lilianae – monocots, monocotyledons,

	monocotyledons
Order	Asparagales
Family	Asparagaceae
Genus	*Sansevieria* Thunb. – *Sansevieria*
Species	*Sansevieria longiflora* Sims – Florida bowstring hemp

Sources: (ITIS, 2007-2010).

2.2.4 Origin and distribution

There is limited information on the origin and distribution of the *Sansevieria longiflora* but *Sansevieria roxburghiana (S. roxburghiana)* Schult. and Schultf. has been described by Prakash *et al.* (2008) to be herbaceous perennial plant with short fleshy stem, occurring in eastern coastal region of India, Sri Lanka, Indonesia and tropical Africa. While *Sansevieria liberica* are widely distributed throughout the tropics (Evans, 2005).

2.2.5 Botanical description

Jeev *et al*, 2012 described *Sansevieria roxburghiana* to belong to Agavaceae family (L), one of hemp species with concave, short petioled leaves that are in part transversely banded with light and dark green, linearly striated with whitish to light green and dark green striations. *Sansevieria longiflora* Sims was described by ITIS, 2007-2010 to consist of four to six leaves on one shoot, lanceolate that grows up to 1.5 cm long and 9 cm across. It is grooved below and flat above. It also has dark green with lighter blotches or irregular bands. Margins are reddish brown and the flowers are around 25 cm long. Inflorescence capitate or densely racemose that is 8-35 cm long (ITIS, 2007-2010). *Sansevieria* is characterized by thick leaves due to a high water content and in some types of *Sansevieria*, based as rosette leaves surround pseudo stem (called a pseudo-stem for surely this plant has no stems) (Yohana, 2012). While other types posse cylindrical leaves which have stiff leaf blade like a sword (Yohana, 2012). Yohana, (2012) described the flower of *Sansevieria* to be sticking in bunches whose length is about 30-80 cm long, light green colour, fragrance with a new bloom in the evening (Yohana, 2012). He further described it seed to be diploid, with two embryos in one seed making it possible to produce two different of new plants (Yohana, 2012). The *Sansevieria longiflora* and its flower is shown in the Plates 2.2 a and b respectively in page 23.

Plate 2.2a: ***Sansevieria longiflora*** **used in the management of wounds in Nigeria.**

Plate 2.2b: The flowers of ***Sansevieria longiflora***

2.2.6 Cultivation

This plant has long rhizomes with rapid rate of growth containing long fibrous roots and are grown ornamentally (USDA, 2008). The plant has a creeping rhizome that grows above the ground or grown in the ground (Yohana, 2012). Single leaf of *Sansevieria*, with its rigid and hard, smooth surface, grow and come together as a root meaning in 2-6 rosette leaves, this gathered at the base of the root grows. All the roots grow from the base of the stem and shaped fibers. Healthy roots are white while the diseased root brown (Yohana, 2012).

2.2.7 Phytochemical properties

The preliminary phytochemical screening of the leaf and rhizome extracts of *Sansevieria roxburghiana* revealed the presence of various chemical compounds such as alkaloids, saponins, flavonoids, phenols, glycosides, proteins, Anthocyanin, betacyanin, phytosterol, steroids, terpenoids, tannins, quinone and carbohydrates some of which have been previously associated with antibacterial activity (Nweze *et al.,* 2004; Philip *et al.,* 2011; Pramoda and Hanumanth, 2015) and this is similar to phytochemical content of related species such as *Sansevieria trifasciata* and *Sansevieria liberica* (Ogukwe *et al.,* 2004; Sunilson *et al.,* 2009; Ikewuchi *et al.,* 2010). Catherine, (2009) reported *Sansevieria roxburghiana* root to contain chemical compounds such as amino acids, vitamins, carbohydrate, minerals, alkaloids, carotenoids, flavonoids (catechins and flavones), phytates, saponins and tannins. Proximate analysis of the leaves of *Sansevieria liberica* contains high crude protein and caloric value, moisture, dry matter, ash, crude lipid, carbohydrate, reducing sugar and crude fibre (Ikewuchi, *et al.,* 2010) while the phytochemical analysis indicates the presence of alkaloids, carotenoids, flavonoids, catechins, flavones, phytates, saponins, and tannins (Ikewuchi, *et al.,* 2010).

Phytochemical screening of *Sansevieria trifasciata* leaves has shown the presence of carbohydrates, saponins, glycosides (Yoshihrio *et al.*, 1996), steroids (Yoshihrio *et al.*, 1997). While Preliminary phytochemical screening of the extracts by Anbu *et al.*, (2009) showed the presence of alkaloids, flavonoids, saponins, glycosides, terpenoids, tannins, proteins and carbohydrates.

2.2.8 Pharmacological and toxicological properties

Amida *et al*. (2007) reported that *Sansevieria liberica* did not produce any visible toxicities or mortality with oral doses up to 20 g/kg within 14 days of single treatment, but intra peritoneal administration caused mortalities with LD_{50} of 668.3 ± 47.6 mg/kg. They also reported that long term administration neither cause mortality nor visible signs of lethality in rats. Furthermore, there is no significant change in the weight of the kidney, liver, heart and spleen, but at 400 mg/kg, there is significant reduction in weight of the lungs, significant increases in the weight of testes, sperm count and motility. Though there are no changes in the sperm head and tail abnormalities, but significant increases in the percentage of normal sperm cells occur (Amida *et al.*, 2007). At 2 g/kg, biochemical parameters like the aspartate transaminase (AST) and alanine transferase (ALT) were not affected, but there was significant increase in alkaline phosphatase (ALP) and uric acid levels. Significant increase and decrease in red blood cell (RBC) and white blood cell (WBC) were recorded, respectively, but no changes in levels of packed cell volume (PCV) and haemoglobin (Hb) (Amida *et al.*, 2007).

2.2.9 Wound healing properties

The leaves of *Sansevieria trifasciata* 'lorentii' can be used to treat swellings, skin diseases such as eczema, toothache, constipation, flu prevention, and detoxification of venomous animals (Yohana, 2012). In Africa, *Sansevieria* has long been used by local residents as anti venom for poisonous snake and insect bites. In some areas of Asian countries, the sap of this plant is used as an antiseptic and leaves used to dress wounds (Yohana, 2012). *Sansevieria* makes wounds to heal faster with minimal side effects (Yohana, 2012). The leaf sap of *S. roxburghiana* is applied directly to infected sores, cuts and grazes (Sardessai *et al.*, 2014).

2.2.10 Antimicrobial effects

Yohana, 2012 reported that *Sansevieria* contain an antiseptic in some parts of the plant, especially the leaves whereas the antimicrobial properties of *Sansevieria roxburghiana* has been widely studied by different researchers. Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity of methanol and acetone extracts of *S. roxburghiana* leaves against Gram-positive bacteria such as *Micrococcus luteus, Bacillus cereus, Enterococcus spp., Staphylococcus aureus,* Gram-negative bacteria such as *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescence, Salmonella typhi, Salmonella paratyphi, Klebsiella pneumoniae, Shigella sonnei* and *Escherichia coli*, fungal strains *Cryptococcus spp.* and *Candida albican* (Philip *et al.,* 2011). Ethyl acetate extracts of rhizomes also exhibited appreciable antimicrobial activity against most of the pathogens tested; however, the leaf extracts exhibited better antimicrobial activity than rhizomes (Philip *et al.,* 2011).

Moideen *et al.,* (2012) reported *Sansevieria roxburghiana* root sap to possess good antibacterial activity on both gram-negative and gram-positive organisms except *Streptococcus faecalis* but has no substantial antifungal properties against the tested fungal strains such *as Aspirgillus niger* and *Candida albicans*. More so in 2015, Pramoda and Hanumanth reported significant antimicrobial activity of *S. roxburghiana* against bacteria such as *Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli*. The antimicrobial activities of plant extracts could be attributed to the presence of different bioactive compounds (Igbinosa *et al.,* 2009) and potent antimicrobial phytochemicals (Reynolds and Dweck, 1999; Alam *et al.,* 2008; Philip *et al.,* 2011).

2.2.11 Anti inflammatory and analgesic properties

The analgesic effects of ethanol and water extracts of *Sansevieria trifasciata* leaves showed dose-dependent and significant increase in pain threshold in tail-immersion test, dose-dependent inhibition of writhing and also a significant inhibition of both phases of the formalin pain test (Anbu *et al.,* 2009). While the ethanol extract significantly reversed yeast-induced fever (Anbu *et al.,* 2009).

Sansevieria trifasciata (Ruscaceae), commonly known as snake plant or mother in-law's tongue, has been traditionally used for the treatment of ear pain, swellings, boils and fever (Anonymous 3, 2005). Fresh leaf sap of *Sansevieria hyacinthoides* (L.) Druce, is applied directly into the eyes of sheep or goats with conjunctivitis while the tuber is used to treat swollen limbs in Tanzania (Bizimana, 1994). Jimuty *et al.,* (2012) also reported the analgesic, cytotoxic and antioxidant activity of the whole plant part of *S. roxburghiana*.

Other studies on *Sansevieria* species have documented its anti inflammatory activity (Mortan, 1981; Da, Silva *et al.,* 2003) analgesic property (Sunilson *et al.,* 2009), antioxidant and antimicrobial activity (Onah *et al.,* 1994; Aliero *et al.,* 2008).

2.2.12 Antitoxic properties

Sansevieria plants have advantages over other plants that are resistant to pollutants because it absorbs it. *Sansevieria* plants contain active ingredients that can reduce pregnanglikoside pollutants into organic acids, sugars, amino acids and some compounds. Research conducted by the United States Space Agency (NASA) shows *Sansevieria* leaves can absorb 107 types of hazardous substances. A plant of *Sansevieria trifasciata* adult lorentii leafy 4/5 strands can refresh the air in the room measuring 20 square meter (Yohana, 2012.)

Britain, France and Japan are now beginning to utilize in-law tongue plant and other plants that can withstand the heat (Yohana, 2012). Sansevieria Lovers Community Advisory Board reported that all parts of this plant can be used as herbal medicine. Some studies even classify this plant as a material medical, because the chemical constituents and pharmacological effects that were tested positive for the leaves, fruits and roots (Yohana, 2012). Kumar and Saritha, (2014) established a link between lead accumulation and antioxidant potential in the species of *Sansevieria roxburghiana* suggesting that the *Sansevieria roxburghiana* was tolerant towards lead (Pb) and can be useful for phytoremediation of lead-contaminated soils.

2.2.13 Antidiabetic properties

The leaves of *Sansevieria trifasciata* 'lorenttii' can be used as an alternative medicine to treat diabetes (Yohana, 2012). Studies on *S. roxburghiana* rhizome demonstrated remarkable antidiabetic activity in streptozotocin induced diabetic rats (Pallab *et al.*, 2010) and antitumor activity (Haldar *et al.*, 2010).

In 2010, Kar evaluated the antidiabetic activity of *Sansevieria roxburghiana* rhizome. In 2012, Qomariyah *et al.* reported decoction of *Sansevieria trifasciata* Prain leaves to lower the blood glucose levels and increase the granule density in the β cells of the islets of Langerhans in alloxan-induced hyperglycemic (diabetic) rats. According to them the *Sansevieria* leaf decoction lowered the blood glucose levels to the same extent as a synthetic hypoglycemic drug (glibenclamide). In 2015, Govindappa reported that *Sansevieria trifasciata* leaves and rhizomes extract decreases blood glucose level of a fasting animal. It has been suggested that in the western part of Nigeria, the rhizomes of *Sansevieria longiflora* is used in the Western part of Nigeria to treat chronic cough and diabetes mellitus while the leaves sap is squeezed on to the wound surface to aid wound healing.

2.2.14 Antineoplastic properties

Ikewuchi, and Ikewuchi, (2011) reported the aqueous extract of the rhizomes of *Sansevieria* to be rich in akuamidine, voacangine, sitosterol and tannic acid. All of these compounds have established antineoplasmic and anticarcinogenic properties (Dillard and German, 2000; Evans, 2005). Some have immunomodulatory, while some others have hypoglycemic properties (Ikewuchi, and Ikewuchi, 2011).

2.2.15 Medicinal or traditional uses

The leaves sap of *Sansevieria intermedia* is used as bait to kill snakes, the leaves are squeezed and the juice is applied on the opening of the hole where snakes reside (Kipkore *et al*. 2014). In Nigeria, the leaves and roots of *Sansevieria liberica* are used in traditional medicine for the treatment of asthma, abdominal pains, colic, diarrhea, eczema, gonorrhea, hemorrhoids, hypertension, menorrhagia, piles, sexual weakness, snake bites and wounds of the foot (Gill, 1992; Osabohien and Egboh, 2008; Adeyemi *et al.,* 2009). The sedative and anticonvulsant activities of the roots have been studied by Adeyemi *et al*. (2007) while the anti-anaemic activities of the leaves have been reported (Ikewuchi *et al.,* 2010b).

In India, *S. roxburghiana* has been traditionally used for several medicinal purposes. The whole plant is traditionally used for the treatment of asthma, abdominal pains, colic, diarrhoea, hemorrhoids, hypertension, menorrhagia, piles, sexual weakness, wounds of the foot, cough, leprosy, cardiotonic, expectorant, febrifuge, purgative, tonic, in glandular enlargement, rheumatism, nutritional deficiencies and treatment of snake bite (Wadher *et al.,* 1995; Dhiman, 2006; Pulliah, 2006; Khare, 2007; Aneesh, 2009).

Sansevieria leaves is used prophylactically against flu, as astringent so as to reduce the heat, preventing inflammation, treating kidney stones, inflammation of the throat, urine laxative, treating diarrhoea, syphilis, hemorrhoids, headaches and fever (Yohana, 2012).

The medicinal properties of *Sansevieria* species is well documented (Wasciky and Hoehn, 1951; Mimaki *et al.,* 1996a.; Mimaki *et al.,* 1996b). The medicinal uses of *Sansevieria* species include treatment for abdominal pains, ear ache, diarrhoea and hemorrhoids (Van

Wyk *et al.*, 1997; Aliero *et al.*, 2008). Traditionally, in treating ear aches and hemorrhoids, the leaves are heated and the warm juice is squeezed onto the affected area. The leaf sap is applied directly to infected sores, cuts and grazes and is also used to treat fungal and scabies infection (Traditional Medicine Database, 2002; Olivia, 2005).

2.3 Wound Healing

A wound is defined as damage or disruption to the normal anatomical structure and function (Robson *et al.*, 2001; Alastair and Mcnaught, 2011). This can range from a simple break in the epithelial integrity of the skin or it can be deeper, extending into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs and even bone (Alonso *et al.*, 1996).

Despite the fact that the processes of repair begin immediately after an injury in all tissues and that all wounds go through similar phases of healing, specialized tissues such as liver, skeletal tissue and the eye have distinctive forms of regeneration and repair and follow separate pathways (Skover, 1991; Lawrence, 1998; Hart, 2002; Toy, 2005).

2.3.1 Classification of wounds

Different criteria is used in classifying wounds (Robson *et al.*, 2001), such criteria include etiology, degree of contamination, morphological characteristics and communication with hollow or solid organs (Bischoff *et al.*, 1999; Komarcevic, 2000; Robson *et al.*, 2001). Wounds can be classified into contusions, abrasions, avulsions, lacerations, cuts, stab wounds, crush wounds, shot wounds and burns on the basis of etiology (Lazurus *et al.*, 1994; Bischoff *et al.*, 1999; Robson *et al.*, 2001; Broughton *et al.*, 2006), while according to the degree of contamination, wounds are classified into: aseptic wounds (bone and joint operations); contaminated wounds (abdominal and lung operations); and septic wounds (abscesses and bowel operations) (Komarcevic, 2000; Vanwijck, 2001; Broughton *et al.*, 2006). Wounds may also be referred to as closed, where the underlying tissue has been traumatized with the skin intact or as open, where the skin layer has been damaged with the underlying tissue exposed (Degreef, 1998; Bischoff *et al.*, 1999; Komarcevic, 2000 Attinger *et al.*, 2006). Wounds can also be clinically categorized as acute and chronic according to their time frame of healing (Lazurus *et al.*, 1994; Bischoff *et al.*, 1999; Robson *et al.*, 2001).

2.3.1.1 Acute wounds: Wounds that proceed normally by following a timely and orderly healing pathway with the end result of both functional and anatomical restoration are classified as acute wounds (Velnar *et al.*, 2009). The time course of healing usually ranges from 5 - 30 days. Acute wounds can be acquired as a result of traumatic loss of tissue or a surgical procedure (Lazurus *et al.*, 1994; Robson *et al.*, 2001).

2.3.1.2 Chronic wounds: Chronic wounds represent a major health burden to the patient, physician and impact upon global health resources (Ud-Din and Ardeshir, 2014). They are wounds that fail to progress through the normal stages of healing hence, cannot be repaired in an orderly and timely manner in less than 42 days (Lazurus *et al.*, 1994; Robson *et al.*, 2001; Singh *et al.*, 2004; Fonder *et al.*, 2008). The healing process is either incomplete or disturbed by various factors, which prolong one or more stages in the phases of haemostasis, inflammation, proliferation or remodeling (Velnar *et al.*, 2009). These factors include infection, tissue hypoxia, necrosis, exudates, excess levels of inflammatory cytokines, diabetes, vascular insufficiency, age and nutritional deficiencies (Vanwijck, 2001; Fonder *et al.,* 2008). Chronic wounds may also result from various causes such as naturopathic, pressure, arterial and venous insufficiency, burns and vasculitis (Degreef, 1998; Komarcevic, 2000).

2.3.2 Wound healing time

Time required for complete regeneration differs in different tissues (Velnar *et al.,* 2009). Some wounds may heal within few days while in others, it may take up to a year or more to heal completely (Flangan, 2000; Komarcevic, 2000; Richardson, 2004). A completely healed wound is defined as one that has been returned to a normal anatomical structure, function and appearance of the tissue within a reasonable period of time (Velnar *et al.*, 2009). Shailajan and Deepti (2016) described the number of days required for falling off the dead tissue remnants without any residual raw wound as the period of epithelialisation.

2.3.3 Mechanisms of wound healing

2.3.3.1 Primary Intention: this is the way by which clean surgical wounds and recent traumatic injuries are managed (Kylie, 2013). The edges of the wounds are approximated with sterile strips, glue, sutures and/or staple (Kathryn, 2014a). There is minimal loss of tissue and scarring (Kylie, 2013).

2.3.3.2 Delayed Primary Intention: this is the surgical closure of a wound 3 -5 days after the thorough cleansing or debridement of the wound bed (Kylie, 2013). It is used to manage traumatic wounds and contaminated surgical wounds (Kylie, 2013).

2.3.3.3 Secondary intention: healing of wounds occurs slowly by granulation, contraction and re-epithelialisation which results in scar formation. It is commonly used to manage pressure injuries, leg ulcers and dehisced wounds (Kylie, 2013).

2.3.3.4 Skin Graft: this is the removal of partial or full thickness segment of epidermis and dermis from its blood supply and transplanting it to another site to speed up healing and reduce the risk of infection (Kylie, 2013).

2.3.3.5 Flap: this is a surgical relocation of skin and underlying structures to repair a wound (Kylie, 2013).

2.3.4 The wound healing process

Healing is a complex process involving co-ordinated interactions between diverse immunological and biological systems (Velnar *et al.*, 2009). It involves a cascade of carefully and precisely regulated steps and events that correlate with the appearance of various cell types in the wound bed during distinct phases of the healing process (Glat and Longaker, 1997; Hunt *et al.*, 2000; Attinger *et al.*, 2006; Broughton *et al.*, 2006). Degreef (1998); Hunt *et al.*, (2000); Vanwijck (2001); Attinger *et al.*, (2006) and Broughton *et al.*, (2006) described the sequence of four time-dependent phases of acute tissue repair to include coagulation and haemostasis phase, inflammatory phase, proliferative phase and remodeling phase.

2.3.4.1 Coagulation and Haemostasis phase: As blood spills into the site of injury, the blood components and platelets come in contact with exposed collagen and other extracellular matrix component which triggers the release of clotting factors from the platelets and the formation of a blood clot, consisting of fibronectin, fibrin, vitronectin and thrombospondin (Pool, 1977; Skover, 1991; Lawrence, 1998; Robson *et al.,* 2001). The blood clot and platelets trapped within it are not only important for haemostasis, but also provides a provisional matrix for cell migration in the subsequent phases of the haemostatic and inflammatory phases (Velnar *et al.,* 2009).

Following activation of platelet by thrombin, thromboxane or adenosine diphosphate (ADP), platelets undergo a change in morphology and secrete the contents of their alpha and dense granules (Zarbock *et al.,* 2007). Activated platelets adhere and clump at sites of exposed collagen to form a platelet plug and temporarily arrest bleeding (Zarbock *et al.,* 2007). Platelets have a crucial role in wound healing process not only they are essential for clot formation, but also produce multiple growth factors and cytokines which continue to regulate the healing cascade (Smyth *et al.,* 2009). Over 300 signalling molecules have been isolated from activated platelets, which influence and modulate the function of other platelets, leukocytes and endothelial cells (Smyth *et al.,* 2009). The main actions of platelet-derived molecules are listed in Table 2.2.

2.3.4.2 Inflammatory phase: The key aim of this stage of wound healing is to prevent infection Regardless of the etiology of the wound, the mechanical barrier which was the frontline against invading microorganisms is no longer intact (Alastair and Mcnaught, 2011). The humoral and cellular inflammatory phase follows coagulation and haemostasis phase with the aim of establishing an immune barrier against invading micro-organisms (Velnar *et al.,* 2009). It is divided into two separate phases, an early inflammatory phase and a late inflammatory phase (Hart, 2002).

Table 2.2. The growth factors involved in wound healing

Factor	Source	Function (s)
TGF-α	Macrophages Platelets	Formation of granulation tissue Stimulates proliferation of epithelial cell and fibroblasts
TGF-β	Platelets Neutrophils Macrophages Fibroblasts	Chemotaxis Transdifferentiation of fibroblasts to myofibroblasts Collagen matrix construction Stimulates angiogenesis Wound contraction Release of other growth factors MMP stimulation
PDGF	Platelets Fibroblasts Endothelial cells Macrophages	Chemotaxis Fibroblast proliferation Collagen deposition
VEGF	Platelets Neutrophils Keratinocytes	Stimulate angiogenesis Neovascularization
Serotonin	Platelets	Vasoconstriction Platelet aggregation Chemotaxis Increase vascular permeability
TNF-α	Platelets	Chemotaxis Nitric oxide release Activation of other growth factors
PGE2	Keratinocytes, Macrophages, Endothelial cells	Vasodilation Platelet disaggregation Increased vascular permeability Pain Fever
Thromboxane A2	Platelets	Platelet aggregation Vasoconstriction
Leukotrienes	Platelets Leukocytes	Increased vascular permeability Chemotaxis Leukocyte adhesion Chemotaxis (neutrophils)
Interleukin-1	Platelets Endothelial cells Lymphocytes	Chemotaxis
Lipoxins	Platelets Leukocytes	Dampen inflammatory response Inhibit chemotaxis (neutrophils)
Interferon-γ	Fibroblasts Lymphocytes	Macrophage maturation Nitric oxide release

KEY: EGF, epidermal growth factor; FGF, fibroblast growth factor; MMP, matrix metalloproteinases; PDGF, Platelet-derived growth factor; PGE2, prostaglandin E2; TGF, transforming growth factor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor. Source: (Alastair and Mcnaught, 2011).

Early inflammatory phase*:* It starts during the late phase of coagulation or shortly thereafter. The complement cascade is activated and molecular events are initiated, leading to infiltration of the wound site by neutrophils, whose main function is to prevent infection (Broughton *et al.,* 2006). The neutrophils start with the critical task of phagocytosis in order to destroy and remove bacteria, foreign particles and damaged tissue so that wound healing can proceed (Robson, 1997; Hart, 2002). The cells that are involved in wound healing are represented in the Table 2.3.

Table 2.3. The Cells that are involved in wound healing

Cell type	Time of action	Function (s)
Platelets	Seconds	Thrombus formation Activation of coagulation cascade Release inflammatory mediators (PDGF, TGF-b, FGF, EGF, histamine, serotonin, bradykinin, prostaglandins, thromboxane
Neutrophils	Peak at 24 hours	prostaglandins, thromboxane. Neutrophils Peak at 24 hours Phagocytosis of bacteria Wound debridement Release of proteolytic enzymes Generation of oxygen free radicals Increase vascular permeability
Keratinocytes	8 hours	Release of inflammatory mediators Stimulate neighbouring keratinocytes to migrate Neovascularization
Lymphocytes	72-120 hours	Regulates proliferative phase of wound healing although exact mechanisms are unclear Collagen deposition
Fibroblasts	120 hours	Synthesis of granulation tissue Collagen synthesis Produce components of extracellular matrix Release of proteases Release of inflammatory mediators

KEY: EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, Platelet derived growth factor; TGF, transforming growth factor. Source (Alastair and Mcnaught, 2011).

The neutrophils are attracted to the wound site within 24 – 36 h of injury by various chemoattractive agents, including tumour growth factor -β (TGF-β), complement components such as C3a and C5a, and formylmethionyl peptides produced by bacteria and platelet products (Robson *et al.,* 2001). Due to alterations in the regulation of surface adhesion molecules, neutrophils become sticky and, through a process of margination, they begin to adhere to the endothelial cells in the post-capillary venules surrounding the wound (Lawrence, 1998; Hart, 2002; Toy, 2005). Then, the neutrophils roll along the surface of the endothelium being pushed forward by the blood flow. These adhesions and rolling mechanisms are mediated by selectin-dependent interactions and are classified as weak attachments (Skover, 1991; Hart, 2002). Chemokines secreted by endothelial cells rapidly activate a stronger adhesion system, which is mediated by integrins (Skover, 1991; Flangan, 2000). Cells stop rolling and migrate out of the venules, squeezing between the endothelial cells by a process known as diapedesis (Skover, 1991; Flangan, 2000; Hart, 2002).

Once in the wound environment, neutrophils phagocytose foreign material and bacteria, destroying them by releasing proteolytic enzymes and oxygen-derived free radical (Skover, 1991; Flangan, 2000; Richardson, 2004). Neutrophil activity gradually changes within a few days of wounding, once all the contaminating bacteria have been removed (Skover, 1991; Hart, 2002). Upon completing the task, the neutrophils must be eliminated from the wound prior to progression to the next phase of healing (Skover, 1991; Hart, 2002). Redundant cells are disposed of by extrusion to the wound surface as slough and by apoptosis, allowing elimination of the entire neutrophil population without tissue damage or potentiating the inflammatory response (Hunt *et al.*, 2000; Hart, 2002). The cell remnants and apoptotic bodies are then phagocytosed by macrophages (Glat and Longaker, 1997; Hunt *et al.,* 2000; Broughton *et al.,* 2006).

Late inflammatory phase: 48 – 72 h after injury, macrophages appears in the wound and continues the process of phagocytosis (Sieggreen, 1987; Witte and Barbul, 1997; Hunt, 1998; Samuels and Tan, 1999; Ennis and Meneses, 2000; Hess, 2000). These cells are originally blood monocytes that undergo phenotypic changes on arrival into the wound to become tissue macrophages. Macrophages are attracted to the wound site by a myriad of chemoattractive agents, including clotting factors, complement components, cytokines such as PDGF, TGF-β, leukotriene B_4 and platelet factor IV, as well as elastin and collagen breakdown products (Velnar *et al.,* 2009). Macrophages have a longer lifespan than neutrophils and continue to work at a lower pH (Pierce *et al.,* 1991a; Ramasastry, 2005). These cells are fundamental for the late stages of the inflammatory response, acting as key regulatory cells and providing an abundant reservoir of potent tissue growth factors, particularly TGF-β, as well as other mediators (TGF-α, heparin binding epidermal growth factor, fibroblast growth factor [FGF], collagenase), activating keratinocytes, fibroblasts and endothelial cells (Glat and Longaker, 1997; Samuels and Tan, 1999; Hunt *et al.,* 2000; Ramasastry, 2005; Broughton *et al.,* 2006). The depletion of monocytes and macrophages from the wound causes severe healing disturbances due to poor wound debridement, delayed fibroblast proliferation and maturation, as well as delayed angiogenesis, resulting in inadequate fibrosis and a more weakly repaired wound (Witte and Barbul, 1997; Ennis and Meneses, 2000; Hess, 2000).

Lymphocytes are the last cells to enter the wound site in the late inflammatory phase (Velnar *et al.*, 2009). They are attracted to the wound site 72 h after injury by the action of interleukin-1 (IL-1), complement components and immunoglobulin G (IgG) breakdown products (Hunt *et al.*, 2000; Hart, 2002; Broughton *et al.*, 2006). The IL-1 plays an important role in collagenase regulation, which is later needed for collagen remodeling, production of extracellular matrix components and their degradation (Sieggreen, 1987; Hunt, 1998; Hart, 2002).

2.3.4.3 Proliferative phase: The proliferative phase starts on the third day after wounding and lasts for about 2 weeks thereafter (Velnar *et al.*, 2009). It is characterized by fibroblast migration and deposition of newly synthesized extracellular matrix, acting as a replacement for the provisional meshwork composed of fibrin and fibronectin (Velnar *et al.*, 2009). At the macroscopic level, this phase of wound healing can be seen as an abundant formation of granulation tissue (Glat and Longaker, 1997; Hunt, 1998). Glat and Longaker, (1997); Hunt, (1998) described the diverse processes that take place in the proliferative phase as follows:

Fibroblast migration: Following injury, fibroblasts and myofibroblasts in the surrounding tissue are stimulated to proliferate for the first 3 days (Witte and Barbul, 1997). They then migrate into the wound, being attracted by factors such as TGF-β and PDGF that are released by inflammatory cells and platelets (Goldman, 2004). Fibroblasts first appear in the wound on the third day after injury and their accumulation requires phenotypic modulation (Velnar *et al.*, 2009). Once in the wound, they proliferate profusely and produce the matrix proteins hyaluronan, fibronectin, proteoglycans, type 1 and type 3 procollagen. All of their products are deposited in the local milieu (Witte and Barbul, 1997; Robson *et al.*, 2001; Ramasastry, 2005). By the end of the first week, abundant extracellular matrix accumulates which further supports cell migration and is essential for the repair process (Sieggreen, 1987; Ramasastry, 2005). Thereafter, fibroblasts change to their myofibroblast phenotype, which contain thick actin bundles below the plasma membrane and actively extend pseudopodia, attaching to fibronectin and collagen in the extracellular matrix (Goldman, 2004; Ramasastry, 2005). Wound contraction, which is an important event in the reparative process that helps to approximate the wound edges, then takes place as these cell extensions retract (Servold, 1991; Greenhalgh, 1998; Baum and Arpey, 2005). Having accomplished this task, redundant fibroblasts are eliminated by apoptosis (Servold, 1991; Greenhalgh, 1998; Baum and Arpey, 2005).

Collagen synthesis: Collagens are synthesized by fibroblasts; they impart integrity and strength to all tissues and play a key role, especially in the proliferative and remodeling phases (Clark, 1993; Greenhalgh, 1998; Baum and Arpey, 2005). Collagens act as a foundation for the intracellular matrix formation within the wound (Velnar *et al.*, 2009).

Angiogenesis and granulation tissue formation: In addition to attracting neutrophils and macrophages, numerous angiogenic factors secreted during the haemostatic phase promote angiogenesis (Pierce *et al.*, 1991a; Servold, 1991; Takeshita *et al.*, 1994). Resident endothelial cells are responsive to a number of angiogenic factors, including FGF, vascular endothelial growth factor (VEGF), PDGF, angiogenin, TGF-α and TGF-β (Velnar *et al.*, 2009). A fine balance is kept by the action of inhibitory factors, such as angiostatin and steroids (Joseph-Silverstein and Rifkin, 1987; Risau, 1990; Ribatti *et al.*, 1991; Zhang *et* *al.,* 1997; Oike *et al.,* 2004). Inhibitory and stimulatory agents act on proliferating endothelial cells directly as well as indirectly, by activating mitosis, promoting locomotion and by stimulating the host cells to release endothelial growth factors (Folkman and Klagsbrun, 1987; Ferrara, 2000). Under hypoxic conditions, molecules are secreted from the surrounding tissue, promoting proliferation and growth of endothelial cells. In response, there is production of proteases by endothelial cells for degradation of the basal lamina in the parent vessel in order to crawl through the extracellular matrix; also there is chemotaxis; proliferation; remodeling and differentiation. FGF and VEGF play central regulatory roles in all of the processes (Folkman and Klagsbrun, 1987; Risau, 1990; Takeshita *et al.,* 1994; Zhang *et al.,* 1997; English *et al.,* 1999; Ferrara, 2000).

Initially, there is no vascular supply in the wound centre, so viable tissue, which is limited to the wound margins, is perfuse by uninjured vessels and by diffusion through undamaged interstitium (Hunt *et al.,* 2000; Phillips, 2000; Robson *et al.,* 2001). Capillary sprouts from the surrounding edges invade the wound clot and, within a few days, a microvascular network composing of many new capillaries is formed (Hunt *et al.,* 2000; Phillips, 2000; Robson *et al.,* 2001).

Chemotaxis is the ability of cells to move along a chemical gradient (Mañes *et al.,* 2000). This biochemical mechanism enables cells to reply properly to environmental stimuli that determine proliferation, differentiation and migration (Mañes *et al.,* 2000). Chemotactic agents act on cell surface receptors to direct the cell migration that is involved in angiogenesis during wound healing (Augustin, *et al.,* 1994; Hsu, 2007). The contributing factors act as mediators for neovascularization and vessel repair at the injury site (Mañes *et* *al.,* 2000). They are also important modulators of cell growth and differentiation, and they include endothelial cell growth factor, TGF-α, VEGF, angiopoietin- 1, fibrin and lipid growth factors (Dejana *et al.,* 1985; Terranova *et al.,* 1985; Grotendorst *et al.,* 1989). Chemotactic activity leads to migration and angiogenesis (Krizbai *et al.,* 2000; Li *et al.*, 2005). As a complex process that involves coordinated changes in cytoskeletal organization, signal transduction and cell adhesion, migration is dependent on the actin-rich network beneath the plasma membrane and is regulated by physical and chemical factors in the vascular system (Li *et al.,* 2005). Regulation is achieved by three mechanisms: chemotaxis (migration towards the concentration gradient of the chemoattractive substance); mechanotaxis (migration induced by mechanical forces); and haptotaxis (migration in response to a gradient of immobilized ligands) (Krizbai *et al.,* 2000; Li *et al.*, 2005). Cellular motility requires three distinct actions: protrusion at the cell front; adhesion, to attach the actin cytoskeleton to the substratum; and finally traction, propelling the trailing cytoplasm forward (Holly *et al.*, 2000; Giannone *et al.,* 2004; Wolgemuth, 2005).

Epithelialisation: Epithelial cells migrate from the edges of the wound soon after the initial insult until a complete sheet of cells covers the wound and attaches to the matrix below (Hunt *et al.,* 2000). A single layer of cells initially forms over the defect, accompanied by a marked increase in epithelial cell mitotic activity around the wound edges (Velnar *et al.,* 2009). When the advancing epithelial cells meet, migration stops and the basement membrane start to form (Baum and Arpey, 2005).

During epithelialisation an embryological process, termed epithelial-mesenchymal transition (EMT), allows epithelial cells to gain motility and travel across the wound surface (Yang and Weinberg, 2008). In wounds that are primarily closed, this phase can be completed within 24 hours. Changes in cytokine concentration result in epithelial cells switching from a motile phenotype to a proliferative one in order to repopulate epithelial cell levels and complete wound repair (De Donatis *et al.,* 2010). In wounds that heal by secondary intention, the area lacking epithelial cells can be large and the wound must contract significantly before epithelialisation can be completed. In some cases this may never occur and skin grafting can be used to cover the defect.

Wound retraction: Wounds begin to contract about 7 days after injury, mediated mainly by myofibroblasts (Hinz, 2006). Interactions between actin and myosin pull the cell bodies closer together decreasing the area of tissue needing to heal. Contraction can occur at a rate of 0.75 mm/day leading to shortened scars (Hinz, 2006). This is influenced by numerous factors including wound shape, with linear wounds contracting fastest and circular wounds the slowest. Disorders of this phase of healing can lead to deformity and the formation of contractures (Hinz, 2006)

2.3.4.4 Remodeling phase: As the final phase of wound healing, the remodeling phase is responsible for the development of new epithelium and final scar tissue formation (Velnar *et al.*, 2009). Synthesis of the extracellular matrix in the proliferative and remodeling phases is initiated contemporarily with granulation tissue development (Ramasastry, 2005). This phase may last up to 1 or 2 years, or sometimes for an even more prolonged period of time (Witte and Barbul, 1997; Ramasastry, 2005). The remodeling of an acute wound is tightly controlled by regulatory mechanisms with the aim of maintaining a delicate balance between degradation and synthesis, leading to normal healing. Along with intracellular matrix maturation, collagen bundles increase in diameter and hyaluronic acid and fibronectin are degraded (Hunt, 1998; Baum and Arpey, 2005). The tensile strength of the wound increases progressively in parallel with collagen collection (Clark, 1993; Baum and Arpey, 2005). Collagen fibres may regain approximately 80% of the original strength compared with unwounded tissue (Clark, 1993; Robson *et al.,* 2001). The acquired final strength depends on the localization of the repair and its duration, but the original strength of the tissue can never be regained (Clark, 1993; Robson *et al.,* 2001). Synthesis and breakdown of collagen as well as extracellular matrix remodeling take place continuously and both tend to equilibrate to a steady state about 3 weeks after injury (Sieggreen, 1987; Clark, 1993). Matrix metalloproteinase enzymes, produced by neutrophils, macrophages and fibroblasts in the wound, are responsible for the degradation of collagen. Their activity is tightly regulated and synchronized by inhibitory factors (Clark, 1993). Gradually, the activity of tissue inhibitors of metalloproteinases increases, culminating in a drop in activity of metalloproteinase enzymes, thereby promoting new matrix accumulation (Clark, 1993; Mulder and Vande Berg, 2002; Toy, 2005). Although the initial deposition of collagen bundles is highly disorganized, the new collagen matrix becomes more oriented and cross-linked over time (Clark, 1993). Its subsequent organization is achieved during the final stages of the remodeling phase, to a greater extent by the wound contraction that has already begun in the proliferative phase (Clark, 1993). The underlying connective tissue shrinks in size and brings the wound margins closer together, owing to fibroblast interactions with the extracellular matrix (Clark, 1993). The process is regulated by a number of factors, with PDGF, TGF-β and FGF being the most important (Pierce *et al.,* 1991b; Clark, 1993). As the wound heals, the density of fibroblasts and macrophages is further reduced by apoptosis (Ganz, 1993; Greenhalgh, 1998). With time, the growth of capillaries stops, blood flow to the area declines and metabolic activity at the wound site decreases (Clark, 1993; Falanga, 1998; Baum and Arpey, 2005), leading to a fully matured scar with a decreased number of cells and blood vessels and a high tensile strength (Falanga, 1998; Hart, 2002; O'Kane, 2002). As the scar matures the level of vascularity decreases and the scar changes from red to pink to grey with time (Alastair and Mcnaught, 2011).

2.3.5 Wound care and management

2.3.5.1 Wound assessment: The first stage of wound management should be a thorough assessment of the wound and the patient (Velnar *et al.*, 2009). The process begins with a diagnosis of the wound's etiology and continues with optimizing the patient's medical condition, particularly blood flow to the wound area (Hunt *et al.*, 2000; Attinger and Bulan, 2001; Dissemond and Goos, 2004). An acute wound in a stable patient with normal blood flow should heal successfully if appropriate care is given (Velnar *et al.*, 2009). The correct approach to treating wounds should effectively assist the healing process and it can have an important impact on the final clinical outcome (Velnar *et al.*, 2009). Physiological, endocrine and nutritional support at a clinical level significantly influence repair and, without them, wound healing often fails completely (Hunt, 1998; Bischoff *et al.*, 1999; Attinger *et al.*, 2006).

2.3.5.2 Wound Cleansing: Wound cleansing may be advocated to remove contaminants like visible debris in wounds, exudates, remnants of dressing material or prior to obtaining a microbiology swab (Kathryn, 2014c). Frequent washing of wounds is however unnecessary and undesirable (Kathryn, 2014c).

2.3.5.3 Cleansing Solutions: In the past wounds were cleansed with antibacterial solutions. Studies comparing the effectiveness of antibacterial solutions to tap water, normal sodium chloride 0.9% and distilled water have found no difference in lowering bacterial count and no increased incidents of infection (Dire and Welsh 1990; Rodeheaver *et al*. 1982). Antiseptic solutions have been reported to cause tissue damage and hinder the healing process and are unlikely to be effective (Hellewell *et al.* 1997).

2.3.5.4 Methods of Cleansing: Irrigation is the cleansing mechanism recommended for removal of contaminants (Miller and Glover 1999). Scrubbing causes pain and local tissue oedema, which decreases host defences. Vigorous cleansing may however be necessary, in some instances, to remove grease and dirt from traumatic wounds (Miller and Glover 1999).

2.3.5.5 Wound lavage and irrigation: This is the lavage of micro-organisms, dead tissue and foreign bodies, which can further decrease tissue bacterial counts (Velnar *et al.,* 2009). Commonly, a bacitracin solution is used (Velnar *et al.,* 2009). In contrast, Patzakis1975, recommended irrigation with large quantities of saline. Low pressure irrigation only removes contamination on the surface (Patzakis, 1975). Although high pressure irrigation reduces bacterial colonization of the wound and the frequency of infections, a high pressure jet can damage fine tissues and push dirt particles deeper into the wound or even into the bone (Fildes *et al.,* 1991). Irrigation with at least 7psi of pressure is needed to flush out any bacteria in a wound (Stevenson *et al.,* 1976). High-pressure irrigation, however, may injure adjacent healthy tissue and cause lateral spread of the irrigating fluid, with resultant postoperative edema. Wounds can be effectively cleansed with ordinary tap water (Valente *et al.*, 2003). Potent antibacterial agents like hydrogen peroxide, povidone-iodine, and alcohol are unnecessary and will destroy healthy tissue (Valente *et al.*, 2003).

2.3.5.6 Wound debridement: Debridement accelerates wound healing and different techniques exist (Hunt *et al.*, 2000; Dissemond and Goos, 2004). Correct debridement helps to convert a chronic wound into an acute one, which can then progress through the normal stages of healing (Greenhalgh, 1998; Feliciano, 2002; Mulder and Vande Berg, 2002; Dissemond and Goos, 2004). The accumulation of devitalized tissue in the wound promotes bacterial colonization and impairs the body's ability to fight infection, thereby preventing complete repair of the wound (Robson *et al.*, 2001). Hence, the aim of debridement is to remove ischaemic and necrotic tissue, which presents potential for infection and contamination of the tissue by bacteria and foreign bodies (Dissemond and Goos, 2004).

Scrubbing with a saline-soaked sponge is a very effective way of removing bacteria, proteinaceous coagulum and debris (Rodeheaver *et al.*, 1975). Scrubbing is best reserved for highly contaminated wounds with embedded particles however it can significantly damage healthy tissue and widen the area of injury (Rodeheaver *et al.*, 1975).

Nonselective or mechanical debridement may include any one or a combination of dry-to-dry, wet-to-dry, and/or wet-to-wet dressing changes; Dakin's solution or hydrogen peroxide; and hydrotherapy or high-powered wound irrigation. Non-selective debridement is used for wounds with large amounts of necrotic tissue and debris. Once granulation tissue begins to develop, a more selective form of debridement should be used and this can be surgical or sharp, autolytic, enzymatic, and mechanical (Attinger and Bulan, 2001; Clerici *et al.*, 2010). The type of debridement method employed depends upon many factors, including the size, position, and type of wound, efficiency of debridement method, pain management, exudate levels, risk of infection, and the cost of the procedure (Sibbald *et al.*, 2000).

Surgical or sharp debridement, which is thought to be selective, may induce damage to viable tissue, and bleeding is likely (Nazarko, 2015; Woo *et al.*, 2015). Some level of autolytic debridement, which is natural and highly selective process by which endogenous proteolytic enzymes break down necrotic tissue, occurs in all wounds (Atkin, 2014; Nazarko, 2015; Woo *et al.*, 2015). Autolytic debridement may not take place fast enough to encourage rapid wound healing and closure, hence it is used in combination with another method of debridement (Nazarko, 2015; Woo *et al.*, 2015). Enzymatic debridement is another highly selective method which uses naturally occurring proteolytic enzymes that are manufactured by the pharmaceutical and healthcare industry specifically for wound debridement (Atkin, 2014; Nazarko, 2015; Woo *et al.*, 2015). Mechanical debridement is a nonselective, physical method of removing necrotic tissue and debris from a wound using mechanical force (Atkin, 2014; Nazarko, 2015; Woo *et al.*, 2015). This debridement method is generally easy to perform and is more rapid than autolytic and enzymatic debridement. However, this nonselective method can damage healthy granulation tissue both in the wound bed and at the margins of the wound thus causing significant discomfort to the patient and compromising complete wound healing and closure (Atkin, 2014; Nazarko, 2015; Woo *et al.*, 2015).

2.3.5.7 Wound treatment: Treatment strategies available for chronic wounds include; compression bandaging (Amsler *et al.*, 2009), wound dressings (Carter *et al.*, 2010), negative pressure wound therapy (Armstrong *et al.*, 2011), ultrasound (Cullum *et al.*, 2010), debridement (Dumville *et al.*, 2009) and skin substitutes (Greaves *et al.*, 2013), which can be expensive, time consuming and may be slow to demonstrate any positive results (Ud-Din and Ardeshir, 2014).

2.3.6 Wound healing and hydration

Hydration is the single most important external factor responsible for optimal healing (Atiyeh and Hayek, 2005). Benefits of moist wound healing include; faster rate of wound healing (Beam, 2008) promote epithelialisation rate (Ågren *et al.*, 2001; Eaglstein, 2001; Wigger-Alberti *et al.*, 2009) and dermal/wound bed healing responses, like cell proliferation, extracelluelr matrix synthesis (Dowsett and Ayello, 2004; Leung *et al.*, 2010; Korting *et al.*, 2011; Mosti, 2013), reduced scarring (Tandara *et al.*, 2007; O'Shaughnessy *et al.*, 2009; Mustoe and Gurjala, 2011; Hoeksema *et al.*, 2013), retention of growth factors at wound site (Attinger *et al.*, 2006; Powers *et al.*, 2013; Hack *et al.*, 2014), lower rate of wound infection (Lawrence, 1994; Kannon and Garrett, 1995), reduced pain perception (Wiechula, 2003; Metzger, 2004; Coutts *et al.*, 2008; Leaper *et al.*, 2012), and enhanced autolytic debridement (Gray *et al.*, 2010; King *et al.*, 2014a).

Landmark studies by George Winter in the 1960s showed that wounds exposed to the air and allowed to dry tend to heal slowly with poor cosmesis when compared with wounds that heal in a moist environment (Winter, 1963; Winter and Scales, 1963). The examination of tissue biopsies from these preclinical studies highlighted that re-epithelialisation of 'dry' wounds was impaired leading to a delay in the healing response and suggested that the physical barrier of the dry eschar tissue was an important determinant for the delayed healing response (Hinman and Maibach, 1963; Winter, 1963). Thus, there is a clear dependency on adequate hydration if optimal healing is sought (Winter, 1963; Winter and Scales, 1963; Bishop *et al.*, 2003).

Ousey *et al.*, 2016 reported that the deleterious effects of maceration on wounds and peri-wound skin is as a result of the presence of damaging biological components fluids that cause maceration and not the presence of water. Also a number of studies have indicated that a wound that is overly hydrated may not result in tissue damage (Svensjö *et al.*, 2000; Vranckx *et al.*, 2002), and rather suggest that a wound bathed in a hyper-hydrated environment may benefit from the advantages of moist wound (Junker *et al.*, 2013).

2.3.7 Current technologies, modalities, and therapies in wound healing

Some of the most successful current therapies in the last decade can be categorized into one of the following four categories: stimulated endogenous, pharmaceutical, surgical, and topical (Erica and Christie, 2016).

2.3.7.1 Stimulated Endogenous Therapies: Stimulated endogenous therapies include the use of additional growth substances (like growth factors, collagen, and cytokines) to stimulate wound healing (Song *et al.*, 2010; King *et al.*, 2014b; Teng *et al.*, 2014; You and Han, 2014).

Mesenchymal stem cells (MSCs) are responsible for the repair response of tissue wounds (Maxson *et al.*, 2012). They function by recruiting cells to and secreting growth factors at the site of injury (Maxson *et al.,* 2012). Growth substance stimulation can also improve wound healing by supplementing the amount of growth factors in the localized area, where cells are able to receive the nutrients needed for maturation and proliferation (Robson and Smith, 2001).

Some studies have shown the positive effects of adding recombinant human Platelet derived growth factor (rhPDGF), fibroblast growth factor (FGF), or epidermal growth factor (EGF) to wounds (Grazul-Bilska *et al.,* 2003). Multiple novel delivery systems, including adenovirus and slow-releasing polymers are being investigated as growth factor delivery systems with varying success (Jia *et al.,* 2015). Some of the most promising growth factors that require further clinical testing are VEGF, bFGF, and GM-CSF (Enestvedt *et al.,* 2008; Tang *et al.,* 2014; Jia *et al.,* 2015). PDGF-BB has already been approved by the U.S. Food and Drug Administration (FDA) and is currently used in the treatment of ulcers (Agren *et al.,* 1999; Wollina *et al.,* 2004; Li *et al.,* 2008). Sustained, simultaneous growth factor therapy, or living cell therapy, which is also FDA approved, may be considered as multiple growth factor therapy as both healthy keratinocytes and fibroblasts produce at least 17 different growth factors (Hirt-Burri *et al.,* 2011).

There are a few examples of products currently on the market that utilize topical collagen; these products have shown wound healing improvement by providing a scaffold for newly recruited cells and growth factors to adhere onto while cell populations grow and tissues thicken (Sarabahi, 2012). An important consideration for using collagen products is moisture control (Erica and Christie, 2016). The wound and collagen need a specific amount of moisture at the wound site for adequate healing as too much moisture promotes infection, while too little moisture collapses the fragile fibrin meshwork (Sarabahi, 2012).

2.3.7.2 Pharmaceutical Therapies: The pharmaceutical therapies associated with wound healing revolve around pain and infection mitigation (Erica and Christie, 2016). Pain medication is used to aid in the patients' comfort throughout the scale of injury to recovery and have been shown to slow the healing process (Cheppudira *et al.,* 2013; Akbik *et al.,* 2014). Antiseptics are used preventively to disinfect the area immediately after injury as well as prohibit conditions where bacteria and other microorganisms breed uncontrollably (Cheppudira *et al.,* 2013; Akbik *et al.,* 2014).

2.3.7.3 Surgical Therapies: The two most commonly used surgical procedures that assist in the wound healing process are skin grafting and resurfacing (Jarjis *et al.,* 2015). A skin graft is a patch of skin that is removed by surgery from one area of the body and transplanted to another area (Mackay and Miraliakbari, 2006). Skin resurfacing and body contouring surgery utilizes a laser to modify scar tissue, discoloration, and other skin formation to that of healthy and aesthetically pleasing skin tone and contour (Jarjis *et al.,* 2015). This procedure is usually performed when the healing process is near completion (Jarjis *et al.,* 2015).

2.3.7.4 Topical Therapies: A fourth therapeutic area is topical, such as bandages, dressings, or sealants (Parsons *et al.,* 2005; Dumville *et al.,* 2014). The three major characteristics that all bandages should possess are antimicrobial, biocompatible, and stable (Glass *et al.,* 2014; Webster *et al.,* 2014). These characteristics are most important for the use of negative pressure wound therapy (NPWT) (Wongworawat *et al.,* 2003; Glass *et al.,* 2014; Webster *et al.,* 2014). NPWT is a recently developed therapy that has become mainstream modality for the most severe wounds, such as those occurring in combat, car accidents, or debilitating workplace injury (Morykwas *et al.,* 1997; Weed *et al.,* 2004; Gregor *et al.,* 2008).

Other modalities used include; Topical growth factor application and incisional priming with PDGF or IL-1 to optimize both the cellular and molecular environment, thus decreasing healing time by modifying inflammation and accelerating the proliferative phase (Chen *et al.,* 1995; Braund *et al.,* 2009). Use of electrical field stimulation to optimize the remodeling phase by promoting more efficient fibroblast recruitment and collagen deposition (Alvarez *et al.,* 1983; Goldman and Pollack, 1996; Sosa *et al.,* 2008) and the use of prosthetic materials to favor tissue repair (Szycher and Lee, 1992; Hodde, 2006). Selective healing with gene therapy (Ramelet *et al.,* 2009), though it is still in pre-clinical development.

2.3.8 Important factors affecting wound healing

Numerous local or systemic, physical conditions or chemical agents either enhance collagen remodeling or impair wound healing (Broughton and Rod, 2005). Some of these are oxygen, haematocrit count, steroids and vitamin A, vitamin C, zinc and other mineral, anti-inflammatory agents, smoking, nitric oxide, age, mechanical stress, nutrition, hydration, environmental temperature, hypoxia, ischemia, trauma, foreign bodies, infection, idiopathic manipulation, chemotherapy, radiation therapy, and other systemic problems such as diabetes mellitus, malnutrition, immunodeficiency, or other disease conditions like obesity, cardiovascular disease, cancer, endocrine disorders, small vessel disease, and renal or hepatic failure all delay wound healing (Siana *et al.,* 1992; Bale *et al.,* 2000; Broughton and Rod, 2005; Atkins *et al.,* 2009; Alastair and Mcnaught, 2011; Berlanga-Acosta *et al.,* 2014; Dhall *et al.,* 2014; Kathryn, 2014b; Molnar *et al.,* 2014; Nunan *et al.,* 2014).

A state of oxygen deficiency, hypoxia, may cause an impairment of function and when the cell is unable to extract adequate oxygen, the partial pressure of oxygen within the cell declines, which leads to a reduction in mitochondrial respiration and oxidative metabolism (Lee *et al.,* 2009). Systemic disease can dramatically prolong or interrupt wound healing (Erica and Christie, 2016). Glycosylation in diabetes mellitus impairs neutrophils and macrophage phagocytosis of bacteria thereby, prolonging the inflammatory phase (Berlanga-Acosta *et al.,* 2014). Any of a group of steroid hormones (corticosteroids) either produced within tissue or made synthetically, can blunt the processes of the entire inflammatory phase. Studies also suggest that non steroidal anti inflammatory drugs (NSAIDs) either short-term or long-term have a negative impact on healing (Stadelmann *et al.,* 1998). NSAIDs can interfere with inflammation modulation and inhibit platelet function and, by extension, have the potential to create a chronic wound (Vane 1987; Stadelmann *et al.,* 1998).

2.4 Wound Microbiology

Though most wounds resist invasive infection, they are usually contaminated (Erica and Christie, 2016). When the concentration exceeds 100,000 organisms per gram of tissue or the immune system becomes compromised, infection frequently ensues (Gottrup, 2001).

2.4.1 Bacterial colonization

Wound colonization is the presence of replicating microorganisms adherent to the wound in the absence of injury to the host (Erica and Christie, 2016).The mere presence of bacteria does not always indicate that a wound is infected (Hutchinson and McGuckin, 1992). All chronic wounds are colonized with bacteria, and usually of more than one species (Hutchinson and McGuckin, 1992). When healing progresses normally, these wound inhabitants rarely attract attention. Sometimes chronic wounds which are colonised by bacteria may progress to complete healing without any setbacks whereas, colonised wounds may also become ‘indolent’ (where there is delayed healing) although there may be no visible deterioration (Kathryn, 2014d).

2.4.2 Critical colonization

Critical colonization refers to the point where a wound is unable to maintain a balance between the number of microbes and the defense systems available (White, 2003). Kingsley (2001) incorporates this notion into a wound infection continuum, extending from sterility to infection as shown the Figure 2.1 in page 58.

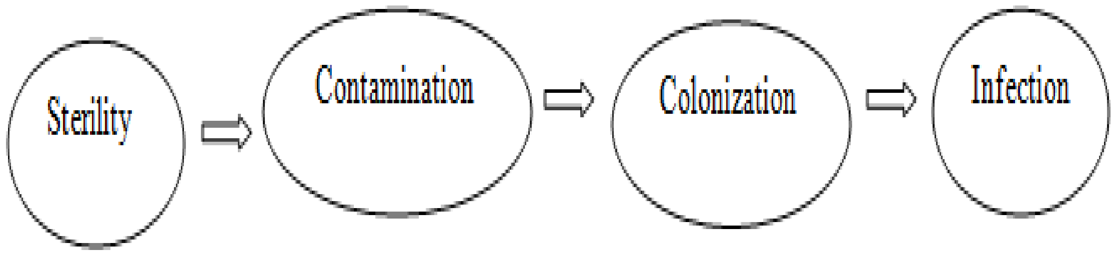

Figure 2.1 Wound infection trend chart

At the point of critical colonization, a wound may not show the multiple classical signs of infection but may cease to heal and become recalcitrant or indolent (Kathryn, 2014d). To differentiate between contamination, colonization and critical colonization is almost impossible as there are often no visible clues (Kathryn, 2014d).

Due to the overuse and resistance problems of systemic antibiotics, researchers have been prompted to revisit the use of antiseptics (Kathryn, 2014d). The antibacterial action of silver and its effect on indolent wounds and burns have been established (Demling and De Santi, 2001; White and Cooper 2001). The use of cadexomer iodine was also suggested in non-healing and infected chronic wounds by Gilchrist (1997), White and Cooper, (2001). However, debridement and antibiotics was considered the mainstays of antibiotic treatment (Erica and Christie, 2016).

2.4.3 Infection

Infection may be defined as the invasion of living cells by micro-organisms (Kathryn, 2014d). The number of microorganisms and their degree of pathogenicity determine the establishment of infection (Kathryn, 2014d). Infection delays healing (Kathryn, 2014d). Misuse or overuse of antibiotics leads to resistance of these and leads to the emergence of new bacterial strains (Bale, Harding and Leaper 2000). Rodeheaver (2001) stated that the single most important parameter to reduce the level of bacterial contamination in the chronic wound is the removal of devitalised tissue (debridement).

Clinical infection is determined by whether the bacteria cause a ‘host reaction’ or not (Kathryn, 2014d). In 1994, Cutting and Harding reported the standards for evaluating infection criteria to include abscess, cellulitis, discharge, delayed healing, discolouration, friable, bleeding granulation tissue, unexpected pain/tenderness, pocketing/bridging at the base of the wound, foul smell and wound breakdown. These was also supported by Gardner *et al.* (2001), who found increasing pain and wound breakdown to be the most sensitive indicators of wound infection.

2.4.4 The microbial flora in wounds

Most of these organisms are normal skin flora like *Staphylococcus epidermidis*, *Corynebacterium* sp., *Brevibacterium* sp., *Proprionibacterium acnes,* and *Pityrosporum* sp. Wound infection refers to the presence of replicating microorganisms within a wound that cause injury to the host, this include, *Staphylococcus aureus*, beta-hemolytic *Streptococcus (S. pyogenes, S. agalactiae), E. coli, Proteus, Klebsiella*, anaerobes, *Pseudomonas, Acinetobacter, and Stenotrophomonas (Xanthomonas)* (Erica and Christie, 2016). Some of the common bacterial strains observed in infected and clinically non-infected wounds are *Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa)*, and β-hemolytic *streptococci* (Sibbald *et al.,* 2000; Li *et al.*, 2010; Percival *et al.,* 2012; Dhall *et al.*, 2014). Of these bacterial strains, *P. aeruginosa* and *Staphylococcus* appear to play an important role in bacterial infection in wounds (Erica and Christie, 2016).

The microbial flora in wounds appears to change over the course of healing (Erica and Christie, 2016). The properties of the wound surface will predetermine which microorganisms will attach, grow and remain components of an early biofilm (Erica and Christie, 2016). Immediately after injury, normal skin flora predominates however, after about 4 weeks facultative anaerobic gram negative rods will colonize the wound (Sibbald *et al.*, 1999). Late in the course of chronic wound, bacterial colonization contains more anaerobes than aerobes though aerobic gram-negative rods also infect wounds at this time (Sibbald *et al.,* 1999; Sibbald *et al.,* 2000; Weed *et al.,* 2004; Folsom *et al.,* 2011; Dissemond, 2014).

2.4.5 Microbial biofilms

The bacteria in a wound occur in the form of biofilms aggregating in complex communities embedded in a self-secreted extracellular polysaccharide matrix (Percival *et al.,* 2014). Biofilms play a significant role in a large number of infections in humans, and due to the intrinsic resistance of these structures to an array of antimicrobial agents and host defense mechanisms, such infections can be difficult to treat effectively (Percival *et al.,* 2014).Colonizing bacteria modify the habitat and create a microenvironment that encourages the attachment and growth of secondary colonizing microorganisms. If unchallenged, and with favorable conditions, a complex community of microorganisms is likely to develop. A microbial biofilm develops the community which ultimately form a more stable polymicrobial "climax community" (Pollock, 1980; Robson, 1997; Sun *et al.,* 2009; Zulkowski, 2013; Pirvanescu *et al.,* 2014).

Antibiotics are used to treat bacterial infections (Sun *et al.,* 2009). However, biofilm-related infections do not yield so easily to this form of treatment, because they provide a protective mechanism that renders bacterial cells less susceptible to both antibiotics and biocides (Mah and Toole, 2001; Malic *et al.,* 2011, Dhall *et al.,* 2014).

2.5 Biochemical Parameters in Wound Healing

2.5.1 Collagen and wound healing

Collagen is the principal building block of connective tissue, accounting for one third of the total protein content of the body (Prockop *et al.*, 1979). It is almost devoid of the sulfur containing amino acids cysteine and tryptophan; instead, collagen contains hydroxyproline and hydroxylysine (Prockop *et al.*, 1979). Collagen has a very complex tertiary and quaternary molecular structure consisting of three polypeptide chains, each chain wound upon itself in a left- handed helix and the three chains together wound in a right-handed coil to form the basic collagen unit (Broughton and Rod, 2005). The polypeptide chains are held in their relative configurations by covalent bonds. Each triple helical structure is a tropocollagen molecule. Tropocollagen units associate in a regular fashion to form collagen filaments; collagen filaments in turn aggregate as collagen fibrils, and collagen fibrils unite to form collagen fibers, which are visible under the light microscope (Broughton and Rod, 2005).

Five types of collagen have been identified in humans on the basis of amino acid sequences and their relative distribution in connective tissues (Table 2.4). Type I collagen is abundant in skin, tendon, and bone (Broughton and Rod, 2005). These tissues account for more than 90% of all collagen in the body (Broughton and Rod, 2005). Normal skin contains Type I and Type III collagen in a 4:1 ratio, the latter mainly in the papillary dermis. In hypertrophic and immature scars the percentage of Type III collagen may be as high as 33% (Bailey *et al.*, 1975).

Table 2.4 Types of collagen found in different tissues

TYPE	STRUCTURE	DISTRIBUTION
I	Hybrid of two chains. Low in hydroxylysine and glycosylated hydroxylysine	Bone, tendon, skin, dentin, ligament, fascia, arteries and uterus
II	Relatively high in hydroxylysine and glycosylated hydroxylysine	Hyaline cartilage, eye tissues
III	High in hydroxyproline and low in hydroxylysine; contains interchain disulfide bonds	Skin, arteries, uterus and bowel wall
IV	High in hydroxylysine and glycosylated hydroxylysine; may contain large globular regions	Basement membrane
V	High in hydroxylysine and glycosylated hydroxylysine; may contain large globular regions	Basement membranes and other tissues

Source: (Broughton and Rod, 2005).

In the inflammatory phase, fibronectin is a major constituent of granulation tissue and seems to promote the adhesion and migration of neutrophils, monocytes, fibroblasts, and endothelial cells into the wound region (Broughton and Rod, 2005). Fibronectin is abundant in the first 24–48 hours of injury and gradually disappears as protein synthesis and chronic inflammation changes become predominant (Broughton and Rod, 2005).

In the fibroblastic or proliferative phase, fibroblasts begin to move into the wound 2 or 3 days after wounding (Grillo, 1963; Stewart *et al.,* 1981). Fibroblasts produce several substances essential to wound repair, beginning with glycosaminoglycans and ending with fibrillar collagen (Delaunay and Bazin, 1964). Glycosaminoglycans are repeating disaccharide units attached to a protein core. Hyaluronic acid is synthesized first, followed in short order by chondroitin-4 sulfate, dermatan sulfate, and heparin sulfate (Bryant, 1977). As these are secreted by the fibroblasts, they are hydrated into an amorphous gel—ground substance—that plays an important role in the subsequent aggregation of collagen fibers (Bryant, 1977).

The conversion of tropocollagen into fibrillar collagen is mediated by the action of two enzymes and calcium (Chapman *et al.,* 1966). Collagen fibrils begin to appear as ground substance accumulates, and over the ensuing 2–3 days are synthesized at a highly accelerated rate. Collagen levels rise continuously for approximately 3 weeks, (Madden and Peacock, 1971) but as increasing quantities of collagen accumulate in the wound, the number of synthesizing fibroblasts begins to decrease, until the rates of collagen degradation and synthesis are equivalent—collagen homeostasis (Madden and Peacock, 1971).

The increase in wound tensile strength that takes place during the fibroblastic phase corresponds to the increasing levels of collagen within the wound (Broughton and Rod, 2005).

In the maturation or remodeling phase, collagen synthesis and degradation are accelerated but no net increase in collagen content (Broughton and Rod, 2005). Large numbers of new capillaries growing into the wound regress and disappear, and collagen fibers initially deposited in a haphazard fashion gradually become more organized and arranged into a pattern determined by local mechanical forces (Broughton and Rod, 2005). During this phase the formerly indurated, raised, and pruritic scar becomes a mature scar, while the wound continues to gain tensile strength (Madden and Peacock, 1971). Most of the embryonic Type III collagen laid down early in the healing process is replaced by Type I collagen, (Bailey *et al.,* 1975) until the normal skin ratio of 4:1 Type I:Type III collagen (Peacock, 1980) is achieved. The macromolecules of the intercellular matrix are progressively degraded, the hyaluronic acid and chondroitin-4 sulfate levels decrease to resemble those of normal dermis, and the water content of the tissues gradually returns to normal (Broughton and Rod, 2005).

2.5.2 Hydroxyproline

The collagen composed of amino acid (hydroxyproline) which is the major component of extra cellular tissue, and it gives strength and support to tissues (Prashanthi *et al.,* 2012). Breakdown of collagen liberates free hydroxyproline and its peptides hence, measurement of the hydroxyproline could be used as an index for collagen turnover (Woessner, 1961;

Prashanthi *et al.*, 2012; Katadj *et al.*, 2016). Wound healing process is characterized by increased hydroxyproline levels in the granulation tissue (Prashanthi *et al.*, 2012).

2.5.3 Hexosamine

By correlating hexosamine content it can be judged how fast the wound heals (Rondle and Morgan, 1955). It is important to note that hexosamine content will increase during wound healing process and decreases when maturation and remodeling phase is attained (Rondle and Morgan, 1955).

2.5.4 Estimation of uronic acid

An increase in uronic acid level in a healing wounds indicate an enhanced synthesis of glycosaminoglycans (Chitra *et al.*, 1998).

2.6 Tensile Strength

Tensile strength is the measurement of its load capacity per unit area (Pirani and Levenson, 1953) or it is the maximum force a material under testing can withstand per unit area (Prashanthi *et al.*, 2012). A wound's breaking strength is defined as the force required breaking it regardless of its dimensions (Pirani and Levenson, 1953). Breaking strength vary from one wound to another whereas, tensile strength is constant for wounds of similar size (Pirani and Levenson, 1953). Collagen fibers are largely responsible for the tensile strength of wounds (Pirani and Levenson, 1953; Bailey *et al.*, 1975). The rate at which a healing wound regains strength varies among species, individuals and different tissues in the same individual (Peacock, 1984). All wounds gain strength at approximately the same rate during the first 14–21 days, but thereafter the curves may diverge significantly according to the tissue involved (Levenson *et al.*, 1965). This is also shown in Figure 2.2.

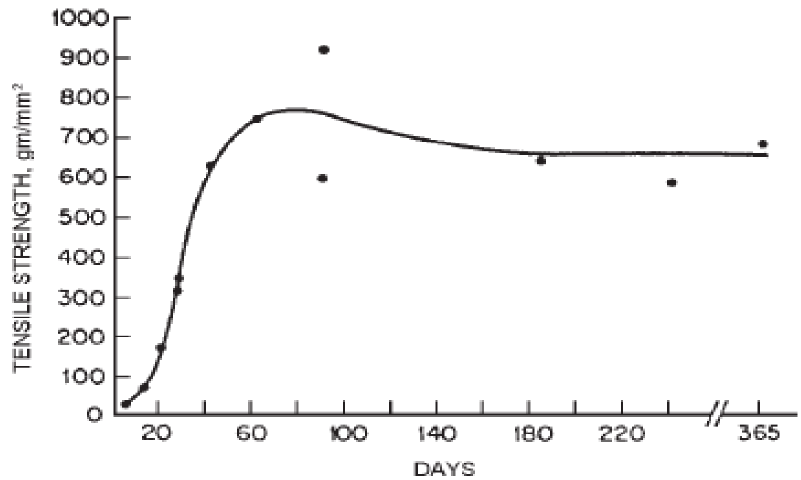

Fig 2.2 The curve for wound tensile strength along with wound healing time

Source: (Broughton and Rod, 2005)

In skin, the peak tensile strength is achieved at approximately 60 days after injury (Levenson *et al.*, 1965). The tensile strength of a wound can never reach that of the original unwounded skin, leveling off at about 80% (Levenson *et al.*, 1965). The increase in hydroxyproline content of the granulation tissue indicates the presence of higher collagen content and its turnover leads to rapid healing with concurrent increase in the tensile strength of the wounds (Prashanthi *et al.*, 2012).

2.7 Pain in Wound Management

Most wounds cause a certain amount of pain but pain management, a key function of all health professionals, is often poorly managed (Casey, 1998). Sometimes pain can be severe and ongoing, such as with chronic wounds, while at other times it may only occur with initial injury, during infection or during dressing change (Kathryn, 2014e).

Patients may experience pain as a result of products or techniques used to cleanse wounds; trauma to the tissues and surrounding skin when products are removed; skin excoriation from exudates or wound drainage; lack of empathy; infection, and poor techniques when using compression bandaging (Kathryn, 2014e). Selecting an inappropriate dressing can result in considerable pain and discomfort (Dealey, 2005). Clinically, pain, like wound types, can be classified as acute or chronic, and can be related to the type of injury, location of the wound, patient perception and previous experience, the healing process, provision of analgesia and approaches to wound management like the choice of dressing (Kathryn, 2014e).

2.7.1 Assessment of pain

Pain should be assessed prior to each dressing change and appropriate action taken to address the cause (Kathryn, 2014e). Patients should be closely observed throughout the dressing procedure for reaction to treatment. Accurate assessment depends on subjective reporting by the patient (Kathryn, 2014e). The patient should be asked if the pain is worse at any particular time or during a particular activity so that analgesic doses can be timed appropriately. It is recommended that a simple visual analogue scale is used (Kathryn, 2014e).

Quantitative pain assessment could be done using equipments like digital force gauge algometer, anaesthesiometer and WAGNER Pain Test ™ Algometer. Pain Test ™ Algometer produced by WAGNER were used to test for pain threshold, tolerance and monitor the treatment progress by pressure algometry at different point of incision. Wagner Pain Test ™ model FKP Algometer is a worldwide preferred threshold and pain tolerance gauge. And pressure algometry has been proven to be a valid measure of localized pain in muscle, joints, tendons, ligaments and bones.

2.7.2 Analgesia

Effectiveness of the analgesia used should be evaluated continuously (Kathryn, 2014e). Failure to achieve pain relief may contribute to the depression and anxiety associated with chronic pain. The type of analgesia to be used depends on the type of wound, whether the wound is acute or chronic, the level of pain reported by the patient; patients' individual circumstances like other medicines, co-morbidities (Kathryn, 2014e). In chronic pain, treatment should be given often enough and regularly to provide continuous pain relief (Kathryn, 2014e).

The use of non-steroidal anti-inflammatory drugs (NSAIDs) like aspirin, ibuprofen, and diclofenac is common in treating minor injuries and in long-term inflammatory conditions. This is due to their action of inhibiting the production of prostaglandins (inflammatory mediators). If wound pain is ongoing it may not be appropriate to use a NSAID due to their side effects (Kathryn, 2014e).

2.7.3 The WHO analgesic ladder

The WHO analgesic ladder forms the basis of many approaches to the use of analgesic medicines. There are three essential steps on this ladder as shown in the Figure 2.3 below:

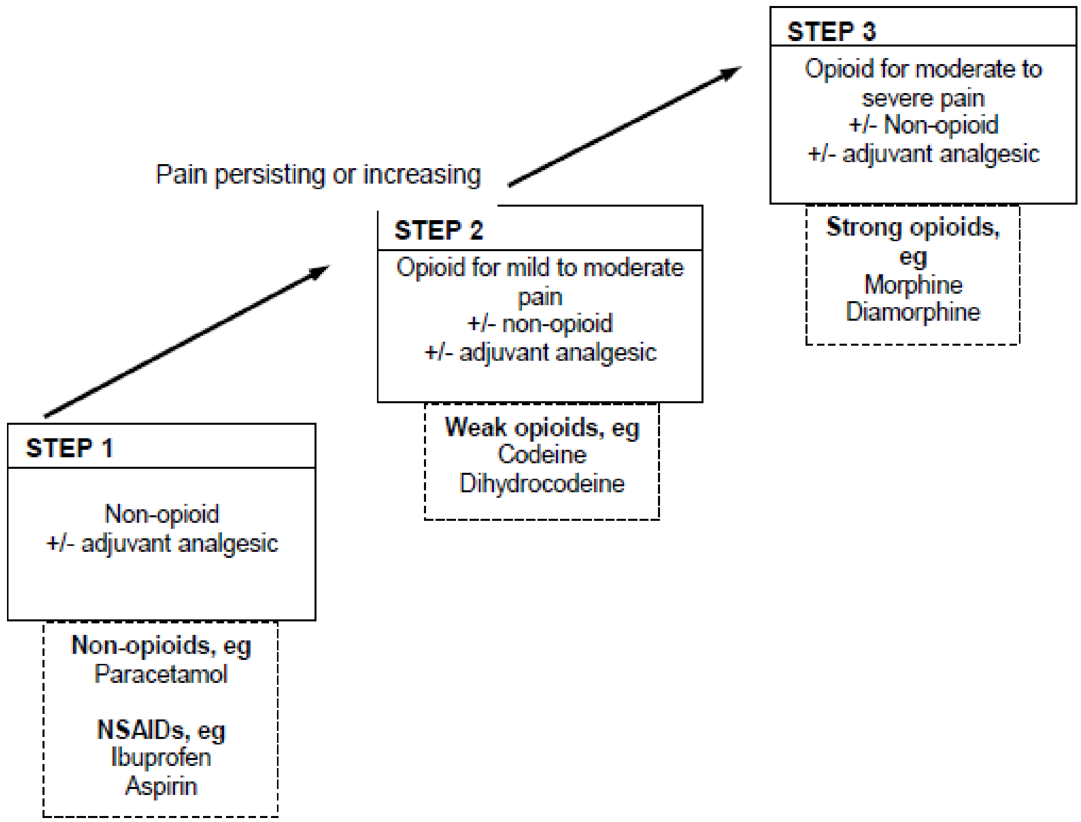

Figure 2.3. The WHO Analgesic Ladder

Source: (WHO, 1996)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Location of Research

The study was carried out in the Department of Veterinary Surgery and Radiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, North-Western Nigeria with geographical coordinates of 11^0 4’0’’ North and 7^0 42’0’’ East (Anon 4, 2013).

3.2 Materials

3.2.1 Equipment

Standard general surgical pack, insulin syringe, xylazin HCl®, ketamine HCl®, algometer, water bath, microtome, glass slide, digital camera, sterile swap stick, petri dish, sample bottle, whatmann No.1 filter paper, centrifuge and graph paper, test tubes, pipettes, colorimeter, caliper, micro pipette. (A complete list is presented in Appendix 5).

3.2.2 Reagents

Hydrochloric acid, chloramine-T, perchloric acid, p-Dimethyl aminobenzaldehyde, Ehrlich’s reagent (1.33 % dimethyl amino benzaldehyde in 1:1 ethanol: concentrated hydrochloric acid mixture), acetylacetone, ethanolic p-methyl aminobenzaldehyde, acetylacetone in sodium carbonate, 95 % ethanol, papain, xylene, ethanol, 10 % formaldehyde in phosphate buffered saline (PSB pH 7.0), paraffin wax, haematoxylene and eosin dye. (A complete list is presented in Appendix 5).

3.2.3 Plant materials

The leaves of the two plants (*Croton lobatus* and *Sansevieria longiflora*) were collected from Obokun Local Government Area of Osun state, South West, Nigeria. The two plants were confirmed in the Herbarium of the department of botany, Ahmadu Bello University, Zaria, and voucher specimen numbers were collected.

3.2.4 Experimental animals

Albino rats (*Rattus norvegicus*) were used for the study.

3.3 Ethical Permission

Ethical permission to use white albino rats for the research was sought for and obtained from the Ahmadu Bello University Committee on Animal Use and Care. The ethical permission number given was ABUCAUC/2016/027.

3.4 Inclusive and Exclusive Factors

Only apparently healthy male and female rats were used in the study.

3.5.1 Traditional mode of application

Leaf paste was prepared from dried *Croton lobatus*. This was applied topically as it is traditionally used (A) while the leaf of *Sansevieria longiflora* was warmed and the sap was squeezed on the wounds (E).

3.5.2 Preparation and administration of aqueous plant extract

Leave of *Croton lobatus* and *Sansevieria longiflora* were harvested and air dried in the shade at room temperature. Dried powders of the plant sample were extracted with sterile distilled water kept on rotary shaker for 24 h. Thereafter, they were filtered through Whatmann No 1 filter paper and centrifuged at 5000G for 15 min. The supernatant were collected and filtered through millipore filter. They were stored at 4° C in air tight bottles as described by Prashanthi *et al*., (2012). Fifty grams (50g) of each extract were dissolved in 100ml of distilled water (500mg/ml) for use. All the wounds created were cleaned and the study group were treated topically with the *Croton lobatus* water extract (B) and *Sansevieria longiflora* water extract (F) once daily as shown in the Table 3.1 (Parekh *et al*., 2005; Ramachandra and Rao, 2008; Oryan *et al*., 2010). Negative control wounds were treated with physiological saline solution (PSS) instead of the extract (C), while positive control wounds were treated with Cicatrin powder (D).

Table 3.1. Experimental design

Code	Plant of study	Number of Animals	Treatment	Amount of treatment
A	*Croton lobatus*	13	*Croton lobatus* (paste)	Liberal
B	*Croton lobatus*	13	*Croton lobatus* (water extract)	2 drops (17 mg)
C	*Croton lobatus*	13	Physiological saline solution (PSS)	2 drops
D	*Croton lobatus*	13	Cicatrin powder	Liberal
E	*Sansevieria longiflora*	13	*Sansevieria longiflora* (sap)	2 drops
F	*Sansevieria longiflora*	13	*Sansevieria longiflora* (water extract)	2 drops (17 mg)

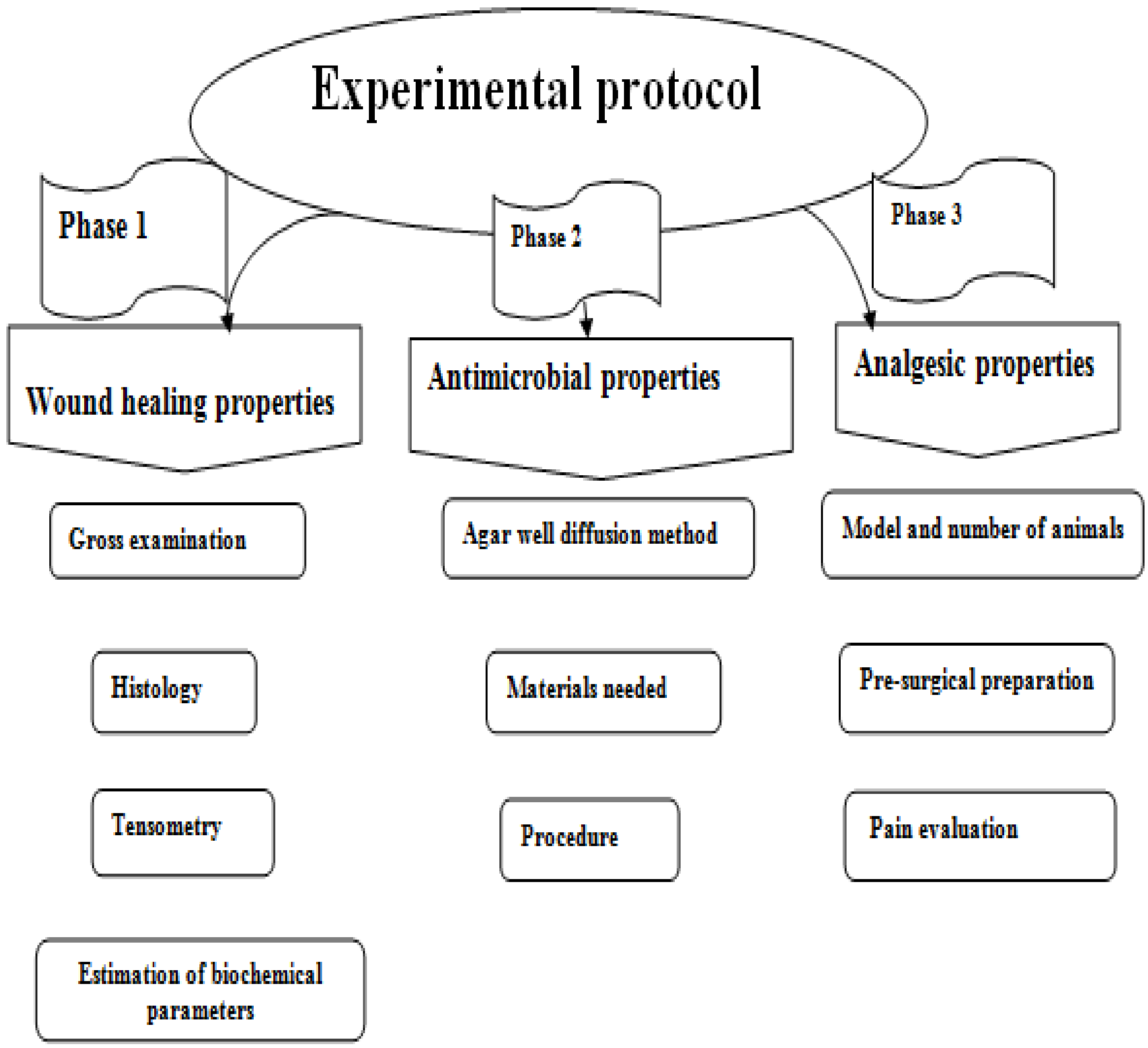

Figure 3.1. Different phases of the research work

3.6 PHASE I (Evaluation of wound healing properties of *Croton lobatus* and *Sansevieria longiflora*)

3.6.1 Experimental design

Twenty six albino rats (male and female) of 22 weeks old, weighing about 190g were selected from an inbred colony obtained from Jos, Plateu State. They were housed in steel cages. The rats were fed with commercial rat feed (prepared from vital feed grower mash) and water *ad libitum*. They were allowed to acclimatize for 2 weeks before the experiment. The animals were divided into 8 groups. Six (6) groups containing 3 animals each were used for gross examination of the wounds, microbial culture, identification and colony forming unit count (CFU). The remaining 2 groups (group 4 and 8) containing 4 animals were used for histology, tensometry and biochemical test as illustrated in the Figures 3.2a and 3.2b.

The animals were anaesthetised by intra muscular injection of ketamine hydrochloride (40 mg/kg) plus xylazine hydrochloride (5 mg/kg) and decontaminated by wiping the whole body with diluted Chlorhexidine Gluconate (Purit®). The dorsal region of each animal was surgically prepared for aseptic surgery and the cleaned dorsal surface of skin was marked with a sterile square shape (1.0 by 1.0 centimeter) stencil. Full-thickness wounds were created by excising the skin flap in an aseptic environment using sterile scissors and forceps. Each wounded animal was housed in a separate cage as described by Oryan *et al*. (2010). The creation of the wounds and rotation of the treatment regimens for each group is as shown in Figures 3.2a and 3.2b.

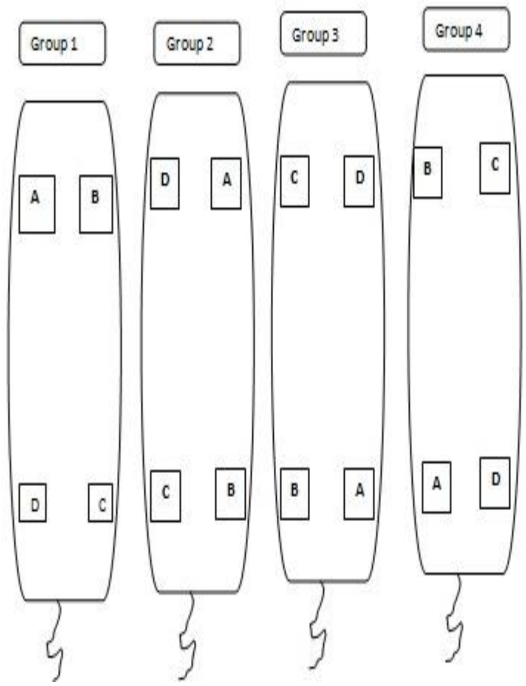

Figure 3.2a. Treatment rotation plan for each group of animals (*Croton lobatus* group)

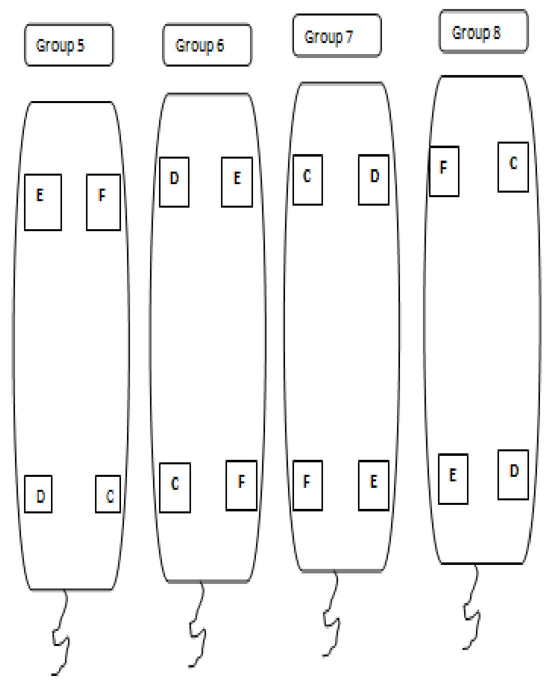

Figure 3.2b. Treatment rotation plan for each group of animals (*Sansevieria longiflora* group)

The wounds were left for 48 hrs to allow for contamination after which they were accessed and samples were collected for culture, identification and CFU counts. Respective treatment was carried out after 48 hrs of wound creation (Table 3.1) and samples were collected at day 1, 3, 5 and 7 after initiation of treatments for CFU counts.

3.6.2 Wound healing experimental protocol

Wound healing was assessed by gross, histopathologic and biochemical examination.

3.6.3 Wound evaluation

3.6.3.1 Gross examination: The wounds were assessed morphologically for presence of pus, development of necrotic tissue, foul odour and exudation. The wound retraction was also measured.

3.6.3.2 Determination of rate of wound contraction: Modifying the method by Sharma *et al*. (2010), wound contraction was monitored planimetrically on days 2, 4, 6, 8, 10 and 12 post wounding by tracing the wound edges on clean tracing paper which were later read from graph paper. Percentage wound contraction was calculated as follows:

% Wound contraction = $(A_0\text{-}A_t)/A_0 \times 100$

Where A_0 was the original wound area and A_t was the area of wound at day 2, 4, 6, 8, 10 and 12 post wounding. Or using the formula by Sadaf *et al.* (2006) as shown below:

$$\%\ \text{Wound contraction} = \frac{\textit{Initial wound size} - \textit{Specific day wound size}}{\textit{Initial wound size}} \mathbf{x100}$$

3.6.3.4: Determination of rate of wound epithelialisation:

% Wound epithelialisation =

$$\frac{\boldsymbol{Specific\ day\ wound\ size - area\ of\ wound\ left\ uncovered\ with\ epithelium}}{\boldsymbol{Specific\ day\ wound\ size}}\boldsymbol{x100}$$

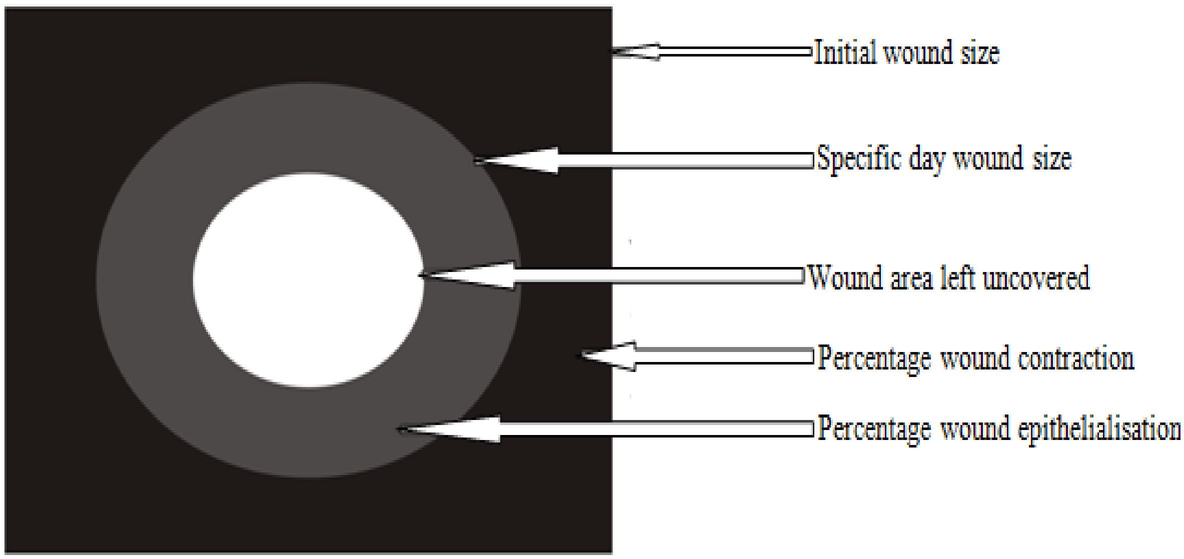

Figure 3.3 Determination of wound contraction and epithelialisation

3.6.4 Histopathological tests

Modifying the methods by Chitra *et al.* (1998); Sumitra *et al.* (2005); and Panchatcharam *et al.* (2006), one animal was sacrificed from each of the *Croton lobatus* groups and *Sansevieria longiflora* (groups 4 and 8) on the day 4, 7, 10 and 13 post wounding and the entire wounds were cut out and samples were collected for histopathological evaluation and granulation tissues for biochemical analysis.

3.6.4.1 Histology procedure: Skin samples from both the wound and comparable adjoining normal skin were fixed in 10 % neutral-buffered formalin. The tissues were mechanically and biochemically stabilized in neutral buffered formalin (10 % formaldehyde in phosphate buffered saline (PSB) pH 7.0). Then the samples were immersed in multiple baths of ascending concentration of ethanol (70%, 80%, 95%, 100%) to dehydrate the tissue, followed by clearing in xylene and immersion in hot molten paraffin wax (impregnation). The tissue were then sectioned into very thin (2-4 μm) sections using a microtome. These sections were placed on a glass slide for staining. Haematoxylin stain was used to study general structure while, Masson green trichrome was used for collagen evaluation (Oryan *et al*., 2010).

The following parameters were evaluated histopathologically: hemorrhage, fibrin deposition, polymorphonuclear cell and mononuclear cell infiltration, reepithelialisation, cornification of the epithelium, fibroblast content, collagen content, revascularizations, necrosis, presence of fibrocytes, maturation and organization of collagen, elastic fibers, fibroblasts and blood vessels.

Acute inflammation scores, chronic inflammation scores, amount of granulation tissue scores, fibroblast maturation scores, collagen deposition scores, neovascularization scores were also evaluated from the histopathological examinations as carried out by Piskin *et al*., (2013).

3.6.4.2 Histological score: Adopting the method of Piskin *et al*. (2013) and Teoh *et al*. (2009), the Abramov's histological scoring system was used in this study. Each parameter was evaluated independently and assigned Abramov's assessment score system. Acute and chronic inflammatory infiltrates, the amount of granulation tissue, and collagen deposition were graded as 0 (none), 1 (scant), 2 (moderate) or 3 (abundant). The fibroblast maturation of granulation tissue were graded as 0 (immature), 1 (mild maturation), 2 (moderate maturation) or 3 (fully matured). Fibroblast maturation was evaluated as to nuclear composition and cytoplasm. Neovascularisation were graded as 0 (none), 1 (up to five vessels per 40 × high magnified area, HMA), 2 (6–10 vessels per HMA) or 3 (more than 10 vessels per HMA).

3.6.4.3 Tensometry (measure of tensile strength): Modifying the methods by Yates *et al.* (2007), bisected biopsies were taken on day 22 after the last wound healed. Tensile strength was measured for each sample using a digital force gauge machine (HF-200). The tensile strength was measured according to established procedures (Hebda *et al.*, 2003), the frozen specimens were trimmed of subcutaneous fat and any muscle that might be taken along with the biopsy. The cross-sectional areas of each specimen were measured with calipers. Then the specimens were clamped on the digital force guage machine and forces (in gram) were exerted until the skin tore. From the measurements recorded by the machine, the tensile strength was calculated as:

Tensile strength (TS) = maximum tensometer reading (converted to g) / Cross-sectional Area (sq-mm)

3.6.5 Biochemical tests

3.6.5.1 Estimation of hydroxyproline: Fifty milligram of tissue were taken into glass tubes and 4 ml of 6N HCl were added to each tube to hydrolyse the tissue sample. The glass tubes were sealed and incubated for 22 hours at 120 ^{0}C. The contents of the tubes were then decanted into a china dish. HCl was removed by evaporation and the residue were dissolved in water and made up to 10 ml using a standard flask. A series of standards were prepared containing 20-200 µg of hydroxyproline with a final volume of 2 ml (Woessner, 1961; Chitra *et al*., 1998).

One millilitre of the hydrolysed tissue samples were used to estimate the contents of hydroxyproline. Hydroxyproline oxidation was initiated by adding 1ml of chloramine-T to each tube in a predetermined sequence. The tube content were mixed by shaking for a few minutes and allowed to stand for 20 min at room temperature. Chloramine-T was destroyed by adding 1 ml of perchloric acid to each tube in the same order as before. The contents were mixed and allowed to stand for 5 minutes. Finally 1ml of p-Dimethyl aminobenzaldehyde solution was added and the mixture was shaken well. Tubes were placed in a 60°C water bath for 20 minutes and then cooled in tap water for 5 minutes. The colour developed was read spectrophotometrically at 557 nm. Hydroxyproline value was determined from the standard curve (Woessner, 1961; Chitra *et al*., 1998).

3.6.5.2 Estimation of hexosamine: Tissue samples (50 mg) were hydrolysed with 2N HCl (5 ml) at 100°C for 6 hours. Hydrochloric acid was removed by evaporation, and the residue were dissolved in water and made up to 10 ml using a standard flask. The method was based on the observation that in alkaline solution at 100 °C, the amino sugars react with acetyl acetone to form chromogenic material which gives a chromophore or chromophores on treatment in acid solution with ethanolic p-dimethyl amino benzaldehyde (Rondle and Morgan, 1955; Chitra *et al*., 1998).

Aliquots containing 10-50 mg hexosamine were treated with 1ml of freshly prepared 2 % acetyl acetone in 0.5 M sodium carbonate in capped tubes and kept in boiling water bath for 15 minutes. After cooling in tap water, 5 ml of 95 % ethanol and 1 ml of Ehrlich's reagent (1.33% Dimethyl amino benzaldehyde in 1:1 ethanol: concentrated hydrochloric acid mixture) were added and mixed thoroughly. The purple red colour developed were read after 30 minutes at 530 nm. Water blank and standard glucosamine solution of various concentrations were treated similarly to get a standard curve (Rondle and Morgan, 1955; Chitra *et al*., 1998).

3.6.5.3 Estimation of total protein: Total protein content of the granular tissue was determined by Bradford (1976) method.

Reagents Required:

Dye Concentrate: One hundred miligram of Coomassie brilliant blue G250 was dissolved in 50 ml of 95 % ethanol. 100 ml of concentrated orthophosphoric acid was added; distilled water was added to a final volume of 200 ml and refrigerated in amber bottles. One volume of concentrated dye solution with 4 volumes of distilled water was mixed for use. Whatman No. 1 paper was used to filter the solution. The protein Standard used was 100 µg/ml in Bovine Serum Albumin (BSA).

Procedure:

Five different volumes which were 0.0, 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard were pipette out in to the series of labeled test tubes. Also, 1 ml of the given sample was pipette out in another test tube. Phosphate Buffer Solution (PBS) was used to make up the volume to 1 ml in all the test tubes. A tube with 1 ml of distilled water served as the blank. Five milliliter (5 ml) of diluted dye solution was added to all the test tubes including the test tubes labeled 'blank' and 'unknown'. The contents of the tubes were mixed by vortexing / shaking the tubes and allow the colour to develop for at least 5 minutes but not more than 30 minutes. The red dye would turn blue when it binds protein. The absorbances at 595 nm were recorded against blank.

3.6.5.4 Estimation of uronic acid: Uronic acid was extracted from granulation tissue as described by Schiller *et al*. (1961). Tissues were briefly digested with papain (10 mg/g wet weight of tissue) in 0.5 M acetate buffer, pH 5.5, containing 0.005 M cysteine and 0.005 M disodium salt of Ethylene diamine tetra acetic acid (EDTA) at 65°C for 24 hours. An aliquot of this digest was used for the estimation of uronic acid by the spectrophotometric method of Bitter and Muir (1962) as follows:

Five milliliter (5 ml) of sodium tetraborate was taken into a test tube and cooled to 4^0C by keeping it in ice bath. One ml of the sample was layered over this and stirred thoroughly with a glass rod. It was allowed to attain room temperature (25^0C) and then heated in a boiling water bath for 10 minutes. After cooling to room temperature, 0.2 ml of carbazole were added and heated again for 15 minutes. in a boiling water bath. It was then cooled after which the optical density was read at 530 nm.

3.7 PHASE II (*in vitro* evaluation of antimicrobial properties)

3.7.1 Evaluation of antimicrobial activity

Agar diffusion method was adopted to determine the antimicrobial properties of the plant samples. Mueller Hinton Agar (MHA) plate was swabbed with respective adjusted 0.5 MF standard micro organisms. Wells of 5 mm in diameter each were created into the agar using cockburer and they were filled with 100 µl of respective test samples (Table 3.2a and 3.2b). The plates were incubated for 18 hrs to 24 hrs after which the diameter of the visible zone of inhibition was measured and compared to the standards.

3.7.2 Materials used

An actively growing broth or streaked plate of a single organism (pure culture of *Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*) Mueller Hinton agar (MHA) plate, antibiotic disk cartridges, CRO 30; E 15; STX 25; OB 5; OX 1; CAZ 10; CN 10; AML 25, *Croton lobatus* (Paste, 0.5 g/ml of water, 0.05 g/ml of water, 0.005 g/ml of water), *Sansevieria longiflora* (Paste, 0.5 g/ml of water, 0.05 g/ml of water, 0.005 g/ml of water). (A complete list is presented in Appendix 5).

3.7.3 Experimental protocol

Three plates of MHA containing *Staphylococcus aureus* inoculate were prepared. *Croton lobatus* extracts of different concentration were added to the first plate (Table 3.2a) while *Sansevieria longiflora* extracts of different concentration were added to the second plate (Table 3.2b). Standard antibiotic impregnated discs were added to the third plate as control. This was repeated for other microorganism.

Table 3.2a Antibiotic sensitivity test for *Croton lobatus*

	Antibiotic disk cartridges	**Code**	**Disk Potency**
1	*Croton lobatus*	CL – P	Paste
2	Croton *lobatus*	CL – 0.5	0.5 g/ml of water
3	*Croton lobatus*	CL – 0.05	0.05 g/ml of water
4	*Croton lobatus*	CL – 0.005	0.00 5g/ml of water
5	Ceftriaxone	CRO 30	30 μg
6	Erythromycin	E 15	15 μg
7	Sulphamethoxazole	STX 25	25 μg
8	Cloxacillin	OB 5	5 μg
9	Oxacillin	OX 1	1 μg
10	Ceftazidime	CAZ 10	10 μg
11	Gentamycin	CN 10	10 μg
12	Amoxycillin	AML 25	25 μg

Table 3.2 b. Antibiotic sensitivity test for *Sansevieria longiflora*

	Antibiotic disk cartridges	**Code**	**Disk Potency**
1	*Sansevieria longiflora*	SL – P	Paste
2	*Sansevieria longiflora*	SL – 0.5	0.5 g/ml of water
3	*Sansevieria longiflora*	SL – 0.05	0.05 g/ml of water
4	*Sansevieria longiflora*	SL – 0.005	0.005 g/ml of water
5	Ceftriaxone	CRO 30	30 μg
6	Erythromycin	E 15	15 μg
7	Sulphamethoxazole	STX 25	25 μg
8	Cloxacillin	OB 5	5 μg
9	Oxacillin	OX 1	1 μg
10	Ceftazidime	CAZ 10	10 μg
11	Gentamycin	CN 10	10 μg
12	Amoxycillin	AML 25	25 μg

3.8 PHASE III (Evaluation of Analgesic Properties)

3.8.1 Incisional wound model

Incisional wound model were used to test for analgesic properties of *Croton lobatus* and *Sansevieria longiflora*. Eight white rats were divided into two groups; *Croton lobatus* was applied to group one while *Sansevieria longiflora* was applied to group two. One centimeter incision wound was created on four different places at the dorsal region of each white rat. Treatment samples were applied topically once daily. Traditional method of application, water extracts, physiological saline solution and xylocaine cream were applied in each of the four wounds. The treatment regimen were rotated as shown in the two groups below (Figure 3.4)

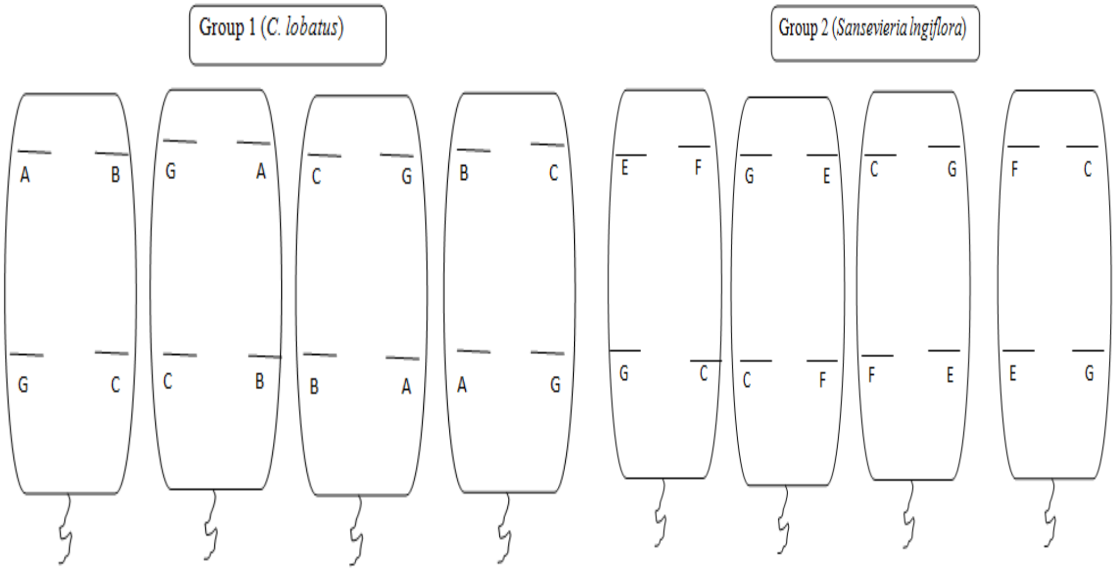

Figure 3.4. Analgesic properties of *Croton lobatus* and *Sansevieria longiflora*

Where A is *Croton lobatus* paste, B is *Croton lobatus* water extract, C Physiological saline solution, G is Xylocaine cream, E is *Sansevieria longiflora* sap, F is *Sansevieria longiflora* water extract.

3.8.2 Postoperative pain evaluation

Response to pain was evaluated with Algometer (digital force gauge machine: HF-200) immediately postoperatively and at 12 hours intervals from day zero (The day on which the wounds were made) till day 5 post incision.

3.9 Data Analysis.

All data were expressed as means ± SEM (standard error of mean). One way and two ways ANOVA were used to analyze different data as appropriate. The Bon ferroni post hoc test was used for multiple comparisons taking time as a factor. Statistical analyses were performed using the Graphpad prism 5.0 for sciences (Graph Pad. 2000). P-Values of less than 0.05 were considered as statistically significant.

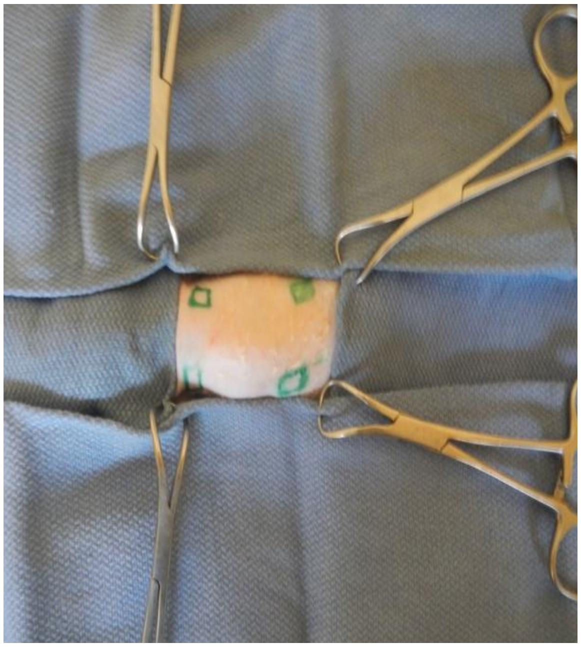

Plate 3.1: Draping of each animal before surgical creation of wounds.

Plate 3.2: Four wounds created on each animal.

Plate 3.3: Animals under recovery post surgery

Plate 3.4: Caging of Animals

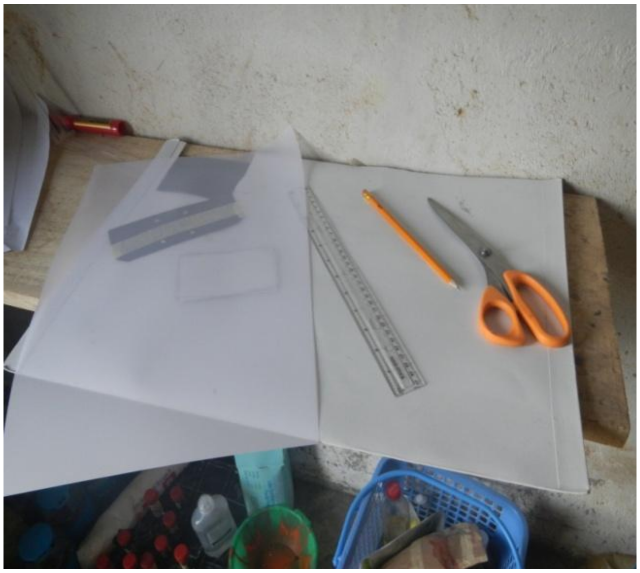

Plate 3.5: Materials used to assess wound contraction; tracing paper, ruler, pencil and scissors.

Plate 3.6: Using the tracing paper to document wound dimension.

Plate 3.7: Digital force gauge in use to evaluate the tensile strength of a healed tissue sample.

Plate 3.8: Digital force gauge in evaluating the pain perception

CHAPTER FOUR

RESULTS

4.1 Laboratory Evaluation

4.1.1 The Voucher Specimen Number

The Voucher Specimen Numbers assigned to the plant samples by the Herbarium of the department of botany, Ahmadu Bello University, Zaria were: *Croton lobatus:* 913 and *Sansevieria longiflora:* 1821.

4.1. 2 Preliminary phytochemical studies

The preliminary phytochemical studies (qualitative analysis) on the leaves of *Croton lobatus* and *Sansevieria longiflora* water extract revealed that they contained carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids, alkaloids and tannins (Table 4.1a).

4.1.3 Quantitative phytochemical studies

The proportion of the alkaloids, flavonoids, saponins, phenols and tannins in the leaves of *Croton lobatus* and *Sansevieria longiflora* water extract is presented in the Table 4.1b.

Table 4.1a Phytochemical constituents of *Croton lobatus* and *Sansevieria longiflora*

S/N	Chemical Test	*Croton lobatus*	*Sansevieria longiflora*
1	Carbohydrates		
	Molisch's test	+	+
	Fehling's test	+	-
2	Cardiac glycoside		
	Kella-killiani test	+	+
	Kedde's test	+	+
3	Free Anthraquinones		
	Borntrager's test	-	-
4	Combined Anthracene		
	Modified Borntrager's test	-	-
5	Saponins		
	Frothing test	+	+
	Haemolysis test	+	+
6	Steroids and Triterpenes		
	Leiberman-Burchards test	+	+
7	Flavonoids		
	Shinoda test	+	+
	Sodium hydroxide test	+	+
8	Tannins		
	Lead sub-acetate test	+	+
	Ferric chloride test	+	-
9.	Alkaloids		
	Mayer's test	+	+
	Dragendorff's test	+	+

Key: +: Present

- : Absent

Table 4.1b Quantitative phytoconstituents of *Croton lobatus* and *Sansevieria longiflora*

Phytochemicals (%)	***Croton lobatus***	***Sansevieria longiflora***
Alkaloids	5.22	3.40
Flavonoids	8.60	10.00
Saponins	3.04	2.80
Phenols	3.40	6.60
Tannins	3.80	2.40

4.2 Wound Healing Properties of *Croton lobatus* and *Sansevieria longiflora*

4.2.1 Wound exudates and debridement

On the third day of wound creation, 75% of the square shaped wounds created had transformed into irregular circular shaped wounds. Scabs were also formed from the wound exudates which were firmly attached to the wound bed. On the fourth day of wound creation, the scabs were dried. The scabs were soaked with physiological normal saline and carefully removed. The amount of exudates and ease of scab removal (debridement) were scored (Appendix 1c and 1d). It was observed that scabs on Cicatrin treated wounds were very sticky, firmly attached to the wound beds and tend to gum to the wound bed during debridement while scabs from other wounds were easily detached. This was also observed on the seventh day after wound creation during second debridement.

Table 4.2a Assessment of the ease of scab removal (debridement) on day 4 and 7 of wounds treated with *Croton lobatus* (n=9)

	A	B	C	D
DAY 4	3 ± (2-3)^{a}	3 ± (3-3)^{a}	2 ± (1-3)^{ab}	0 ± (0-1)^{b}
DAY7	3 ± (2-3)^{a}	3 ± (3-3)^{a}	2 ± (1-2)^{b}	0 ± (0-1)^{b}

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline); D (Cicatrin powder); 0 (poor); 1 (good); 2 (very good) and 3 (excellent).

Table 4.2b Assessment of the ease of scab removal (debridement) on day 4 and 7 of wounds treated with *Sansevieria longiflora* (n=9)

	E	**F**	**C**	**D**
DAY 4	$2 \pm (1\text{-}3)^{a}$	$3 \pm (1\text{-}3)^{a}$	$2 \pm (0\text{-}3)^{ab}$	$0 \pm (0\text{-}1)^{b}$
DAY7	$2 \pm (1\text{-}3)^{a}$	$2 \pm (1\text{-}3)^{a}$	$2 \pm (1\text{-}2)^{ab}$	$0 \pm (0\text{-}1)^{b}$

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline); D (Cicatrin powder); 0 (poor); 1 (good); 2 (very good) and 3 (excellent).

Table 4.2c Evaluation of scab (exudate) from the wounds treated with *Croton lobatus* on day 4 and 7 (n=9)

	A	**B**	**C**	**D**
DAY 4	3 ± (2-3)	2 ± (2-3)	2 ± (1-3)	2 ± (2-3)
DAY7	2 ± (2-3)	2 ± (2-2)	2 ± (2-3)	2 ± (2-3)

[abc]Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline); D (Cicatrin powder); 0 (none); 1 (scanty); 2 (moderate) and 3 (abundant).

Table 4.2d Evaluation of scab (exudate) from the wounds treated with *Sansevieria longiflora* on day 4 and 7 (n=9)

	E	**F**	**C**	**D**
DAY 4	2 ± (2-3)	3 ± (2-3)	2 ± (2-3)	2 ± (2-3)
DAY7	2 ± (2-3)	2 ± (2-3)	2 ± (2-3)	2 ± (2-3)

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline); D (Cicatrin powder); 0 (none); 1 (scanty); 2 (moderate) and 3 (abundant).

4.2.2 Wound contraction

The mean values for percentage wound contraction increased with days in all the treated wounds (*Croton lobatus* paste, *Croton lobatus* water extract, *Sansevieria longiflora* sap, *Sansevieria longiflora* water extract, normal saline and Cicatrin powder) with slight differences (Appendix 1a and Plate 9a to 22b). The highest rate of wound contraction occurred in all the treated groups at day 6 (Table 4.3a and 4.3b). At day 4, the maximum wound contraction was observed with *Croton lobatus* extract (24.54 ± 6.37 %) and *Sansevieria longiflora* extract (27.58 ± 6.49%) while the minimum value was recorded with Cicatrin treated wounds (3.56 ± 5.56%) (Table 4.3a). At day 12 the maximum wound contraction was observed in *Croton lobatus* extract treated wounds (94.10 ± 0.9%) and *Sansevieria longiflora* sap treated wounds (93.27 ± 0.5%) while the minimum value was recorded with normal saline treated wounds (86.50 ± 5.76%) (Table 4.3b).

Table 4.3a Mean values of percentage wound contraction (%) on the day 4, 6, 8, 10 and 12 of wound healing using *Croton lobatus* (n=9)

	A	**B**	**C**	**D**
DAY 4	4.13 ± 3.82^{a}	24.54 ± 6.37^{b}	9.43 ± 8.77^{a}	3.56 ± 5.56^{a}
DAY 6	62.42 ± 3.66^{a}	71.48 ± 3.37^{a}	61.96 ± 5.37^{ab}	51.73 ± 4.96^{b}
DAY 8	82.84 ± 3.32	88.77 ± 1.58	86.06 ± 1.56	81.96 ± 3.04
DAY 10	91.61 ± 1.31	93.89 ± 0.98	90.23 ± 0.96	89.32 ± 1.13
DAY 12	92.29 ± 1.21	94.10 ± 0.90	86.50 ± 5.76	90.00 ± 1.16

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

Table 4.3b Mean values of percentage wound contraction (%) on the day 4, 6, 8, 10 and 12 of wound healing using *Sansevieria longiflora*. (n=9).

	E	**F**	**C**	**D**
DAY 4	25.38 ± 5.51^{a}	27.58 ± 6.49^{a}	16.51 ± 5.25^{ab}	8.86 ± 4.32^{b}
DAY 6	69.80 ± 3.46^{ab}	74.03 ± 4.06^{a}	60.87 ± 6.14^{b}	58.74 ± 3.06^{b}
DAY 8	86.85 ± 1.33	88.13 ± 2.21	79.16 ± 3.62	79.17 ± 2.08
DAY 10	90.97 ± 1.04	90.49 ± 2.58	88.99 ± 1.66	86.31 ± 1.71
DAY 12	93.27 ± 0.50	92.60 ± 1.60	91.38 ± 1.61	89.00 ± 1.09

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.2.3 Wound epithelialisation

The mean values for percentage wound epithelialisation increased with days but with marked differences among the treated wounds (*Croton lobatus* paste, *Croton lobatus* water extract, *Sansevieria longiflora* sap, *Sansevieria longiflora* water extract, normal saline and Cicatrin powder) (Appendix 1b; Table 4.4 and Plate 13a to 22b).

Epithelialisation started at about day 7 and reached its highest rate at day 10. At day 8 the highest wound epithelialisation was observed with *Croton lobatus* paste (62.10 ± 4.93%) while the lowest value was recorded with physiological normal saline treated wounds (35.00 ± 8.01%) (Table 4.4a). At day 12 the highest percentage wound epithelialisation was observed in *Croton lobatus* paste treated wounds (96.67 ± 3.335%) and *Sansevieria longiflora* sap treated wounds (94.64 ± 4.71%) while the lowest value was recorded with Cicatrin treated wounds (60.97 ± 8.45%) (Table 4.4a).

Table 4.4a Mean values of percentage wound epithelialisation (%) on the day 8, 10 and 12 of wound healing using *Croton lobatus* (n=9).

	A	B	C	D
DAY 8	62.10 ± 4.93	59.58 ± 5.62	50.61 ± 9.24	39.55 ± 9.85
DAY 10	82.62 ± 6.01^{a}	84.63 ± 5.15^{a}	76.87 ± 5.56^{ab}	55.52 ± 10.99^{b}
DAY 12	96.67 ± 3.33	96.44 ± 3.56	87.49 ± 4.41	76.43 ± 6.88

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

Table 4.4b Mean values of percentage wound epithelialisation (%) on the day 8, 10 and 12 of wound healing using *Sansevieria longiflora* (n=9).

	E	**F**	**C**	**D**
DAY 8	40.02 ± 8.87	42.72 ± 8.40	35.00 ± 8.01	43.71 ± 6.23
DAY 10	72.23 ± 10.56[ab]	73.31 ± 8.33[a]	58.35 ± 8.68[ac]	43.93 ± 10.87[bc]
DAY 12	94.64 ± 4.71[a]	92.61 ± 4.11[a]	79.97 ± 9.35[ab]	60.97 ± 8.45[b]

[abc]Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

DAY 3

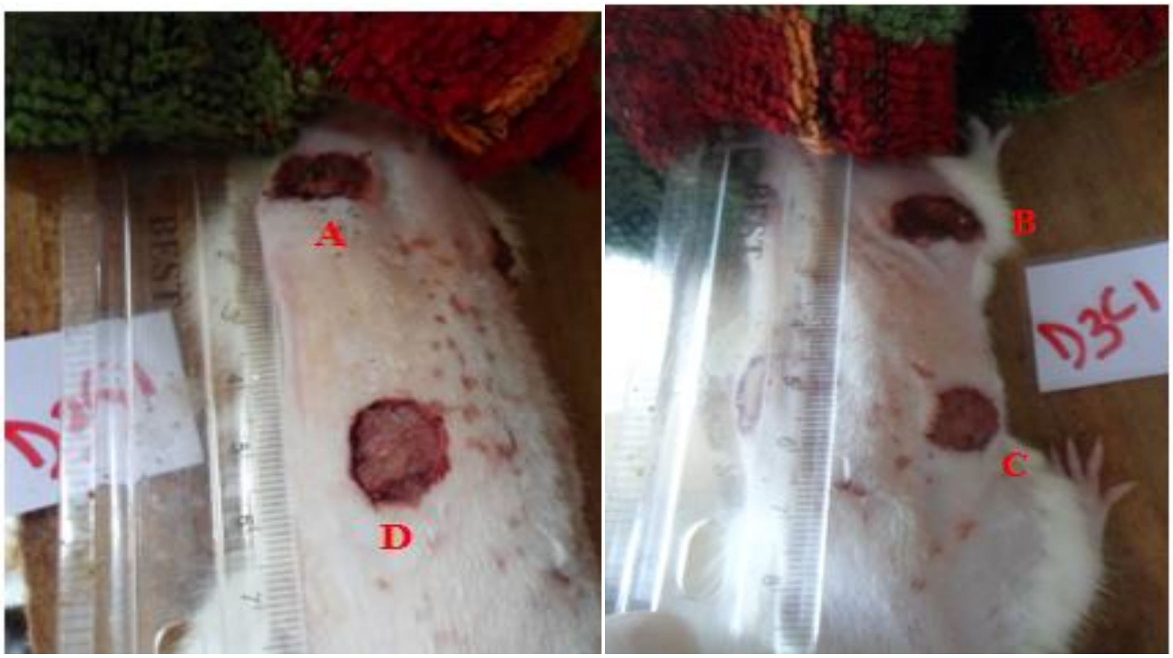

Plate 4.1: Wounds treated with *Croton lobatus* water extracts. Transformation of all the wounds into irregular circular wounds at day 3. (A, *Croton lobatus* paste, B, *Croton lobatus* water extract, C, Normal saline and D, Cicatrin powder).

Plate 4.2: Wounds treated with *Sansevieria longiflora* water extracts. Transformation of the wounds into irregular circular wounds at day 3. (E, *Sansevieria longiflora* sap, F, *Sansevieria longiflora* water extract, C, Normal saline and D, Cicatrin powder)

DAY 11

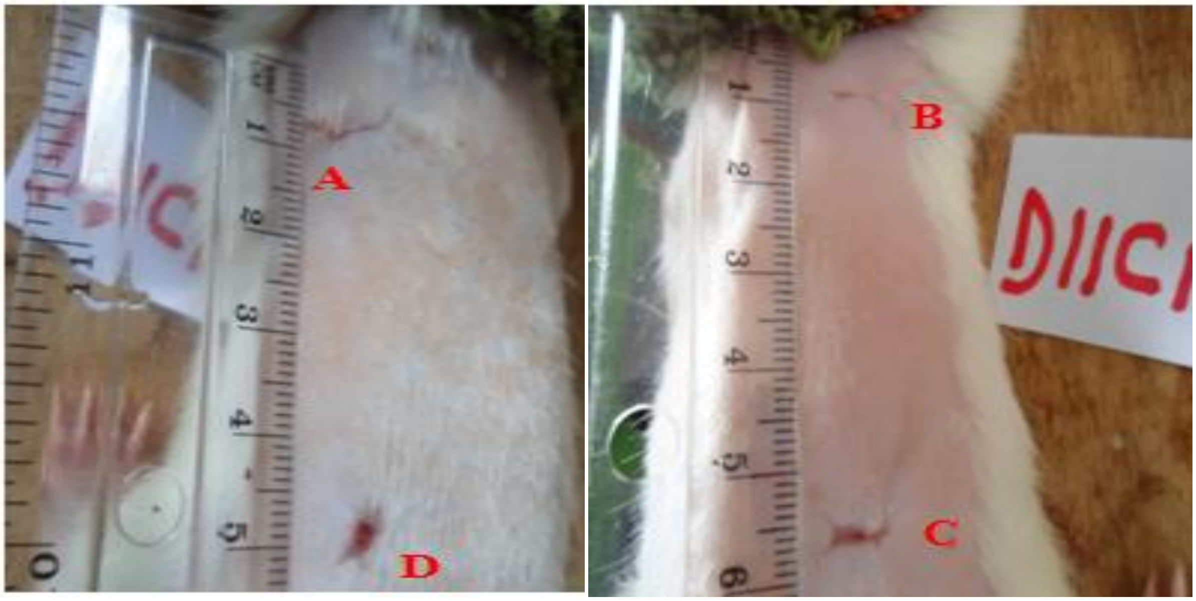

Plate 4.3: Wounds treated with *Croton lobatus* water extracts. The size and epithelialisation of the wounds at day 11 (C and D) while A and B are already healed. (A, *Croton lobatus* paste, B, *Croton lobatus* water extract, C, Normal saline and D, Cicatrin powder)

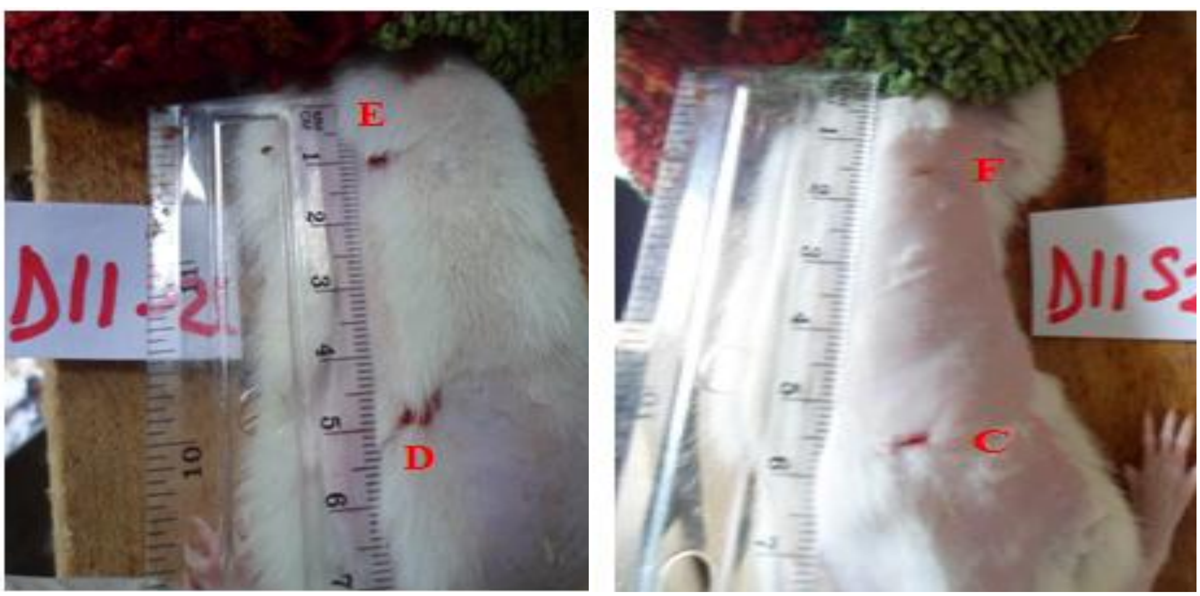

Plate 4.4: Wounds treated with *Sansevieria longiflora* water extracts. The appearance and size of the wounds at day 11. (E, *Sansevieria longiflora* sap, F, *Sansevieria longiflora* water extract, C, Normal saline and D, Cicatrin powder)

DAY 17

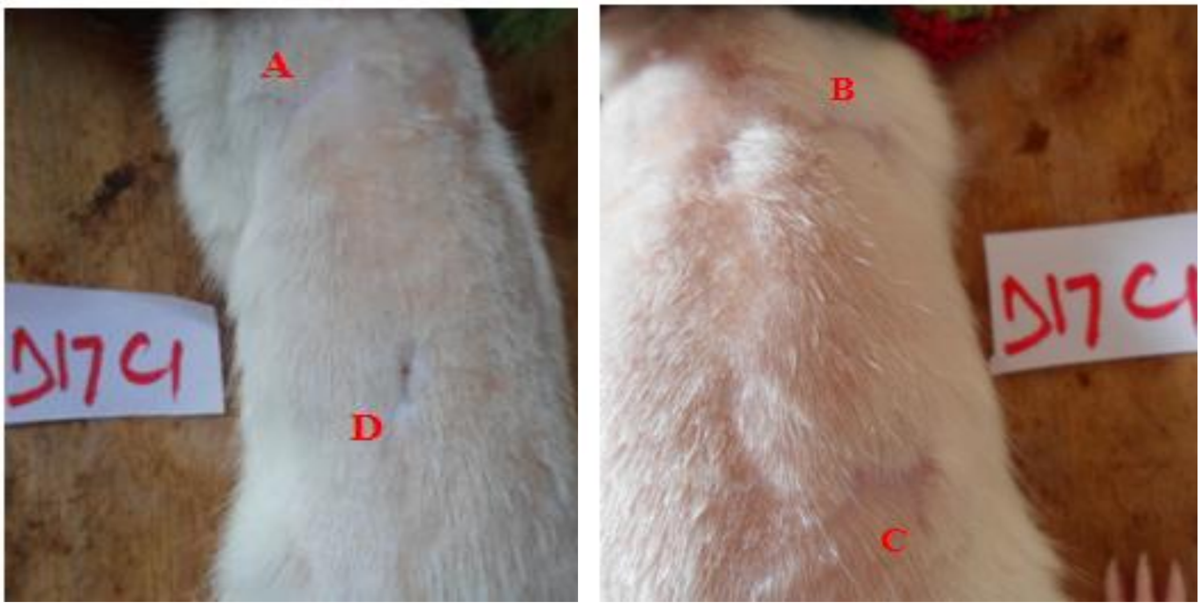

Plate 4.5: Wounds treated with *Croton lobatus* water extracts. The scars formed on the healed wounds at day 17. (A, *Croton lobatus* paste, B, *Croton lobatus* water extract, C, Normal saline and D, Cicatrin powder)

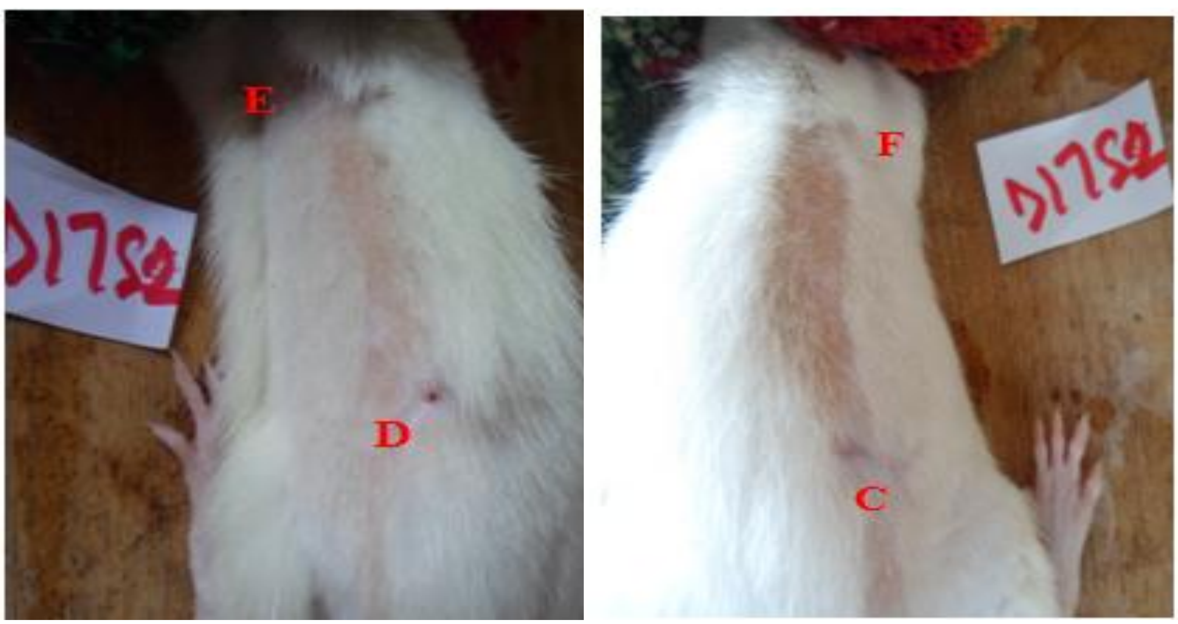

Plate 4.6: Wounds treated with *Sansevieria longiflora* water extracts. The scars formed on the healed wounds at day 17. (E, *Sansevieria longiflora* sap, F, *Sansevieria longiflora* water extract, C, Normal saline and D, Cicatrin powder)

4.2.4 Histology

From the histological studies of the harvested granulation tissues, there was moderate to abundant amount of fibroblast and active fibroblast in the *Croton lobatus* and *Sansevieria longiflora* treated wounds compared to the normal saline and Cicatrin treated wounds in which they were scanty (Table 4.5; Plate 23 and 24). Similarly, collagen deposition was also moderate to abundant in the *Croton lobatus* and *Sansevieria longiflora* treated wounds compared to the normal saline and Cicatrin treated wounds however, fewer polymorphonuclear cells were present. At the 13th day of wound healing, epithelialisation was complete in the *Croton lobatus* and *Sansevieria longiflora* treated wounds with abundant amount of keratinocyte and new vascular blood vessels.

Table 4.5 Histopathology of the granulation tissues

Day	Sample	Fibroblast	Active fibroblast	PMNL	Keratinocyte	Collagen	Revascularization
4	A	3	3	3	1	2	0
4	B	2	3	3	0	2	0
4	C	2	3	2	0	2	0
4	D	2	3	3	0	2	0
4	E	2	3	2	0	2	0
4	F	2	3	3	2	1	0
4	C	2	1	2	0	1	0
4	D	2	1	3	2	1	0
7	A	3	3	1	3	3	0
7	B	3	3	1	3	3	0
7	C	3	1	3	3	2	0
7	D	1	3	3	3	1	0
7	E	3	3	1	3	2	0
7	F	2	3	1	2	2	1
7	C	2	1	3	0	1	0
7	D	1	1	3	0	1	0
10	A	2	3	1	3	2	1
10	B	2	3	1	3	2	1
10	C	2	1	2	1	1	0
10	D	1	1	2	1	1	0
10	E	2	3	1	2	3	0
10	F	2	2	1	2	2	1
10	C	1	1	1	0	1	0
10	D	1	3	3	1	1	0
13	A	2	2	1	2	1	1
13	B	2	2	1	2	1	2
13	C	2	2	1	1	2	1
13	D	1	2	2	1	1	1
13	E	3	1	1	3	3	2
13	F	2	1	1	2	3	1
13	C	2	3	2	1	2	1
13	D	2	2	2	1	2	1

KEY: For Fibroblast, Active fibroblast, PMNL, Keratinocyte and Collagen (0 – None; 1 – Scant; 2 – Moderate; 3 – Abundant) for Revascularization (0 – None; 1 - up to 5 vessles per 40X high magnified area (HMA); 2 – 6 to 10 vessles per 40X high magnified area; 3 - more than 10 vessles per 40X high magnified area); PMNL (polymorphonuclear nuclear leukocyte and inflammatory cell types); A, *Croton lobatus* paste, B, *Croton lobatus* water extract, E, *Sansevieria longiflora* sap, F, *Sansevieria longiflora* water extract, C, Normal saline and D, Cicatrin powder.

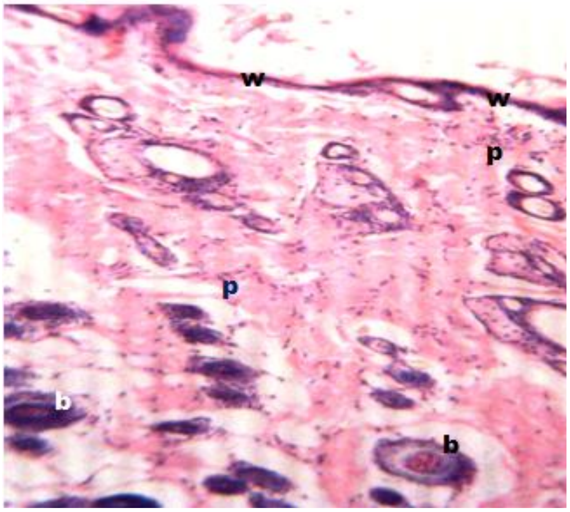

Plate 4.7a: Micrograph of the granulation tissue harvested from the wound treated with *Croton lobatus* paste at day 4 post wounding. w (wound area), b (blood vessel) and p (mixed type inflammatory cells). H&E staining, Mg x 100

Plate 4.7b: Micrograph of the granulation tissue harvested from the wound treated with *Croton lobatus* water extract at day 4 post wounding. w (wound area), b (blood vessel) and p (mixed type inflammatory cells). H&E staining, Mg x 100

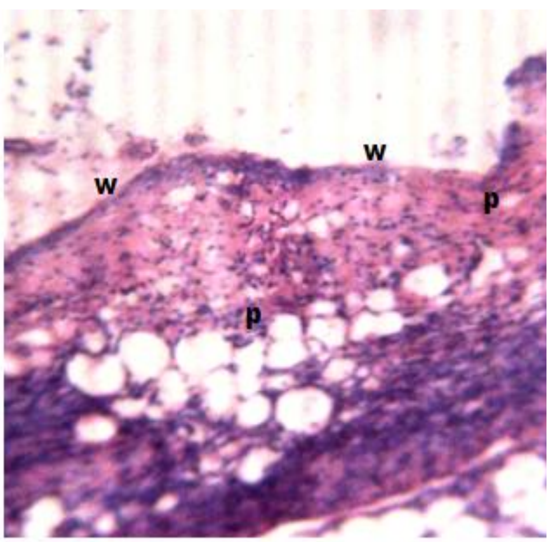

Plate 4.7c: Micrograph of the granulation tissue harvested from the wound treated with n ormal saline at day 4 post wounding. w (wound area) and p (mixed type inflammatory cells). H&E staining, Mg x 100.

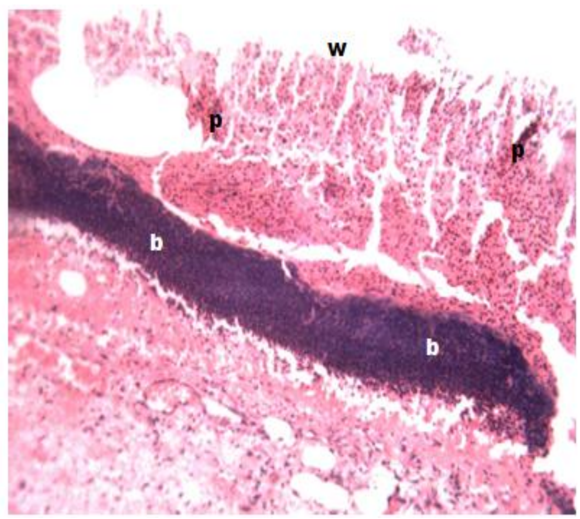

Plate 4.7d: Micrograph of the granulation tissue harvested from the wound treated with Cicatrin powder at day 4 post wounding. w (wound area), b (blood vessel) and p (mixed type inflammatory cells). H&E staining, Mg x 100

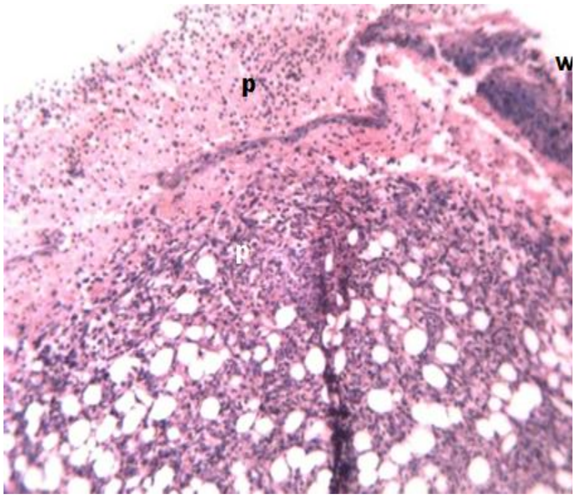

Plate 4.7e: Micrograph of the granulation tissue harvested from the wound treated with *Sansevieria longiflora* sap at day 4 post wounding. w (wound area) and p (mixed type inflammatory cells). H&E staining, Mg x 100.

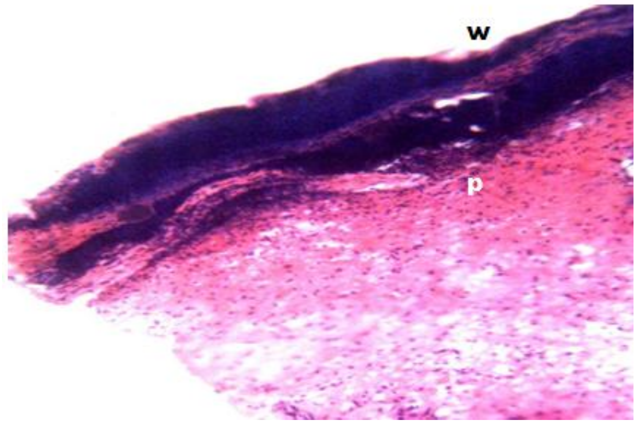

Plate 4.7f: Micrograph of the granulation tissue harvested from the wound treated with *Sansevieria longiflora* water extract at day 4 post wounding. w (wound area), b (blood vessel) and p (mixed type inflammatory cells). H&E staining, Mg x 100.

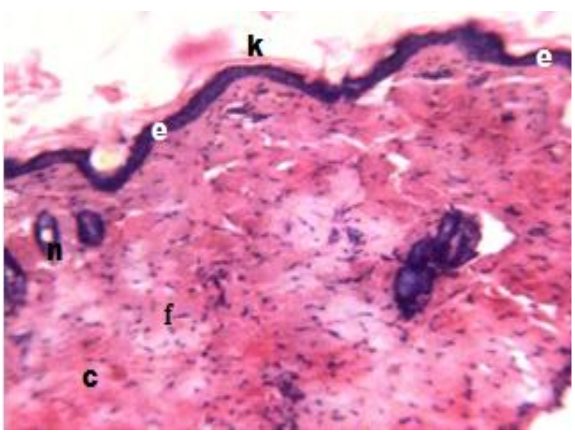

Plate 4.8a: Micrograph of the granulation tissue harvested from the wound treated with *Croton lobatus* paste at day 13 post wounding. f (fibroblast), c (collagen fibres), k (keratinisation), e (epithelialisation), and n (neovascularization). H&E staining, Mg x 100.

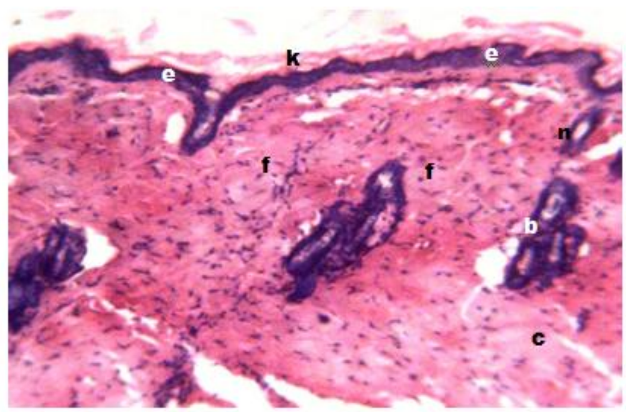

Plate 4.8b: Micrograph of the granulation tissue harvested from the wound treated with *Croton lobatus* water extract at day 13 post wounding. f (fibroblast), c (collagen fibres), b (blood vessel), k (keratinisation), e (epithelialisation), and n (neovascularization). H&E staining, Mg x 100.

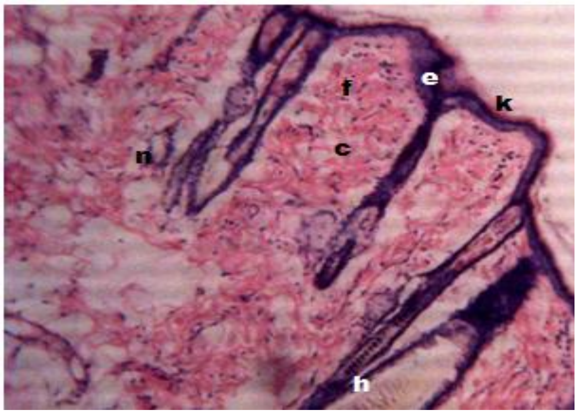

Plate 4.8c: Micrograph of the granulation tissue harvested from the wound treated with normal saline at day 13 post wounding. f (fibroblast), c (collagen fibres), h (hair follicle), k (keratinisation), e (epithelialisation), and n (neovascularization). H&E staining, Mg x 100.

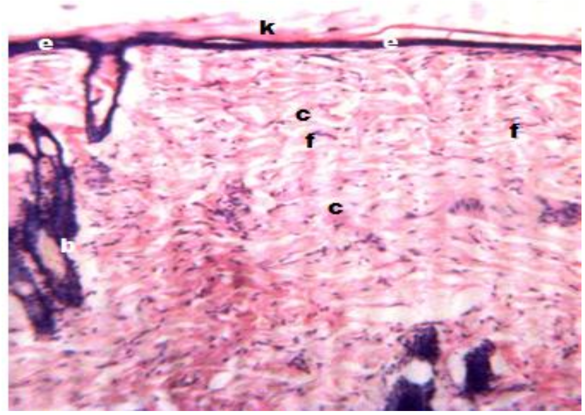

Plate 4.8d: Micrograph of the granulation tissue harvested from the wound treated with Cicatrin powder at day 13 post wounding. f (fibroblast), c (collagen fibres), k (keratinisation), e (epithelialisation), and b (blood vessel). H&E staining, Mg x 100.

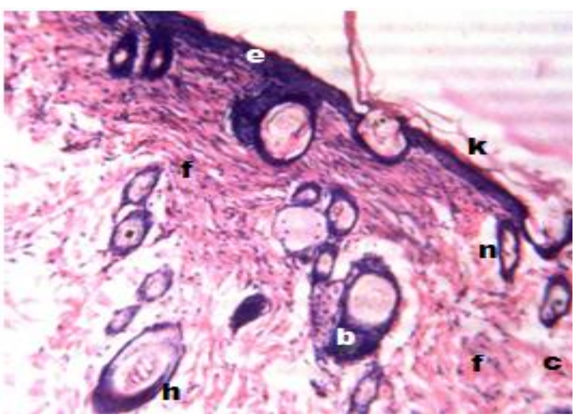

Plate 4.8e: Micrograph of the granulation tissue harvested from the wound treated with *Sansevieria longiflora* sap at day 13 post wounding. f (fibroblast), c (collagen fibres), h (hair follicle), k (keratinisation), e (epithelialisation), b (blood vessel) and n (neovascularization). H&E staining, Mg x 100.

Plate 4.8f: Micrograph of the granulation tissue harvested from the wound treated with *Sansevieria longiflora* water extract at day 13 post wounding. f (fibroblast), c (collagen fibres), k (keratinisation), e (epithelialisation), b (blood vessel) and n (neovascularization). H&E staining, Mg x 100.

4.2.5 Tensometry

During the early phase of wound healing, the tissue samples harvested for tensometry got torn during sample collection, despite extra care that was taken while handling them. Hence, the sample collection for tensometry was delayed till after the last wound healed (Appendix 1f).

The mean value of the tensile strength at day 22 after the last wound healed was higher with the *Croton lobatus* paste (437.40 ± 8.79 g/cm^2) and *Sansevieria longiflora* sap (453.60 ± 129.80); while in Cicatrin treated wounds, the value was 163.00 ± 27.75 g/cm^2 (Table 4.6a and 4.6b).

Table 4.6a Mean values of tensile strength of tissues (g/cm^2) from various healed wounds treated with *Croton lobatus* water extract at day 22 (n=2).

	A	B	C	D
DAY 22	437.4 ± 8.79	384.8 ± 68.5	312.5 ± 3.82	206.5 ± 89.81

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

Table 4.6b Mean values of tensile strength of tissues (g/cm^2) from various healed wounds treated with *Sansevieria longiflora* water extract at day 22 (n=2)

	E	**F**	**C**	**D**
DAY 22	453.6 ± 129.8	341.9 ± 84.06	225.9 ± 33.04	163.0 ± 27.75

[abc]Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.2.6 Estimation of wound healing time

The time at which each wounds healed is presented in the Appendix 1e. Wounds that were treated with *Croton lobatus* paste healed within the shortest period of time (11.0 ± 0.33 days), followed by *Croton lobatus* water extract and *Sansevieria longiflora* sap at (11.22 ± 0.40) and (12.0 ± 0.50) days respectively. Cicatrin treated wounds had the longest period of wound healing (17.56 ± 1.04) days (Table 4.7a and 4.7b).

Table 4.7a Mean value of time (days) it took the wounds treated with *Croton lobatus* water extract to heal (n=9).

A	B	C	D
11 ± 0.33^a	11.22 ± 0.40^a	12.33 ± 0.50^a	17.56 ± 1.39^b
(10-12)	(10-13)	(10-14)	(10-22)

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

Table 4.7b Mean value of time (days) it took the wounds treated with *Sansevieria longiflora* to heal (n=9).

E	F	C	D
12 ± 0.50^{a}	12.5 ± 0.65^{a}	13.75 ± 0.45^{a}	17 ± 1.04^{b}
(10-14)	(10-16)	(12-16)	(13-21)

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.3 Biochemical Effects of the Plant Samples on Wound Healing

4.3.1 Hydroxyproline

Hydroxyproline generally decreased in all the wounds by day 7 post wounding, and increased to its peak at day 10, then declined with slight variation (Appendix 2). The highest hydroxyproline content was recorded with *Croton lobatus* paste and water extract treated wound (Figure 4.1a). For the *Sansevieria longiflora* group, the hydroxyproline content decreased at day 7, followed by increase to its peak at day 10 and then declined. However, *Sansevieria longiflora* sap treatment wound recorded highest content of hydroxyproline (Figure 4.1b).

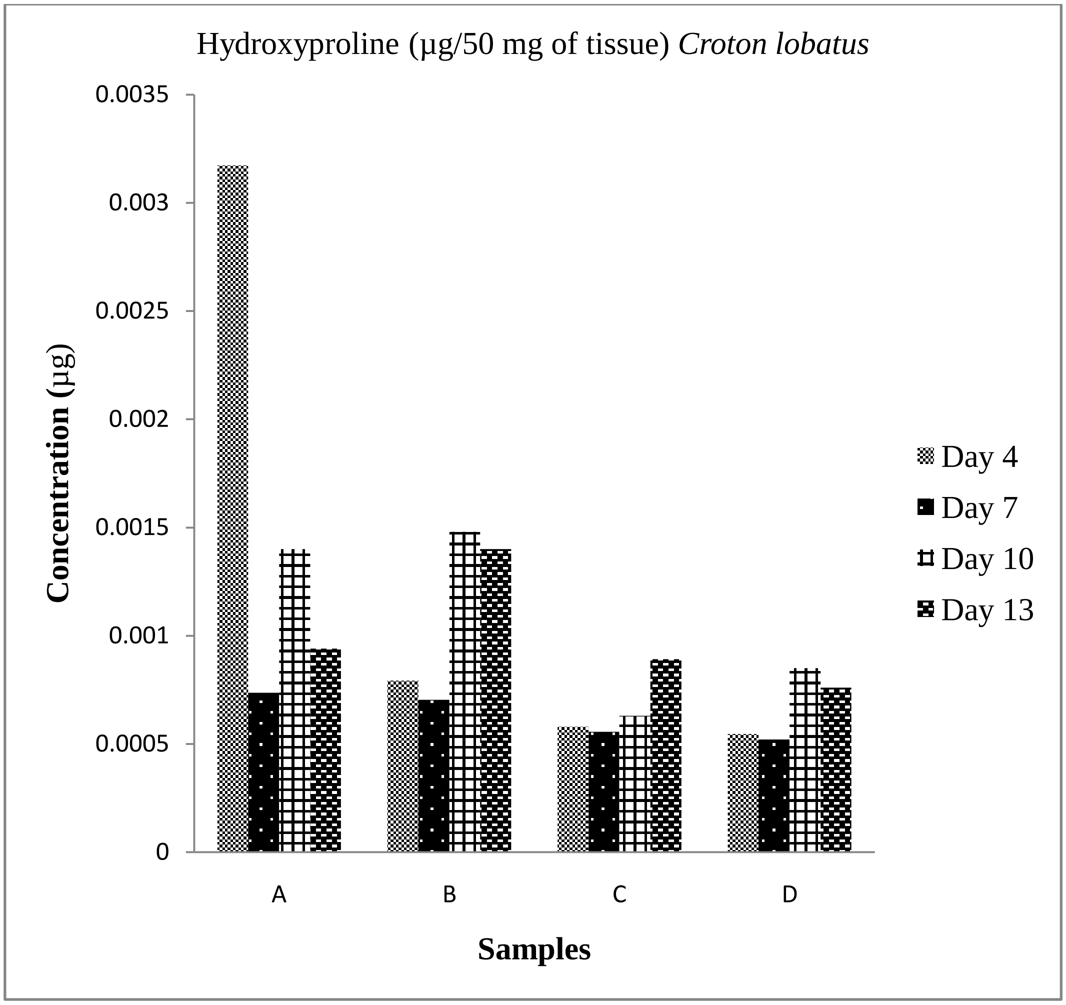

Figure 4.1a: Biochemical estimation of hydroxyproline (µg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (A, B, C, and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

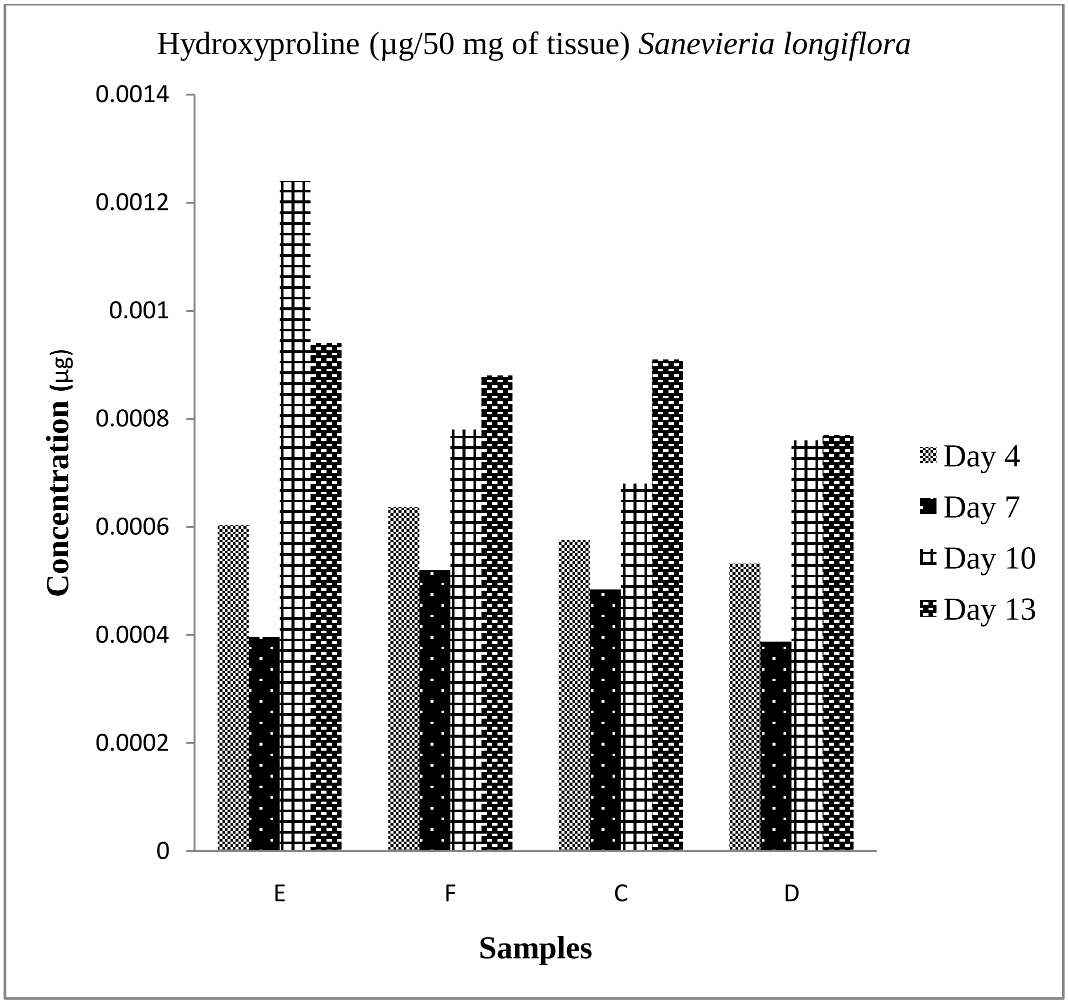

Figure 4.1b: Biochemical estimation of hydroxyproline (µg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (E, F, C and D).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.3.2 Hexosamine

Hexosamine content generally dropped at day 7 and increased to its peak at day 10 after which it declined again (Appendix 2). *Croton lobatus* paste and water extract recorded the highest hexosamine content (Figure 4.2a). For *Sansevieria longiflora* group, hexosamine also followed the same curve by dropping at day 7, increased to its peak at day 10 and then declined. *Sansevieria longiflora* sap treated wound recorded the highest peak value of hexosamine content at day 10 (Figure 4.2b).

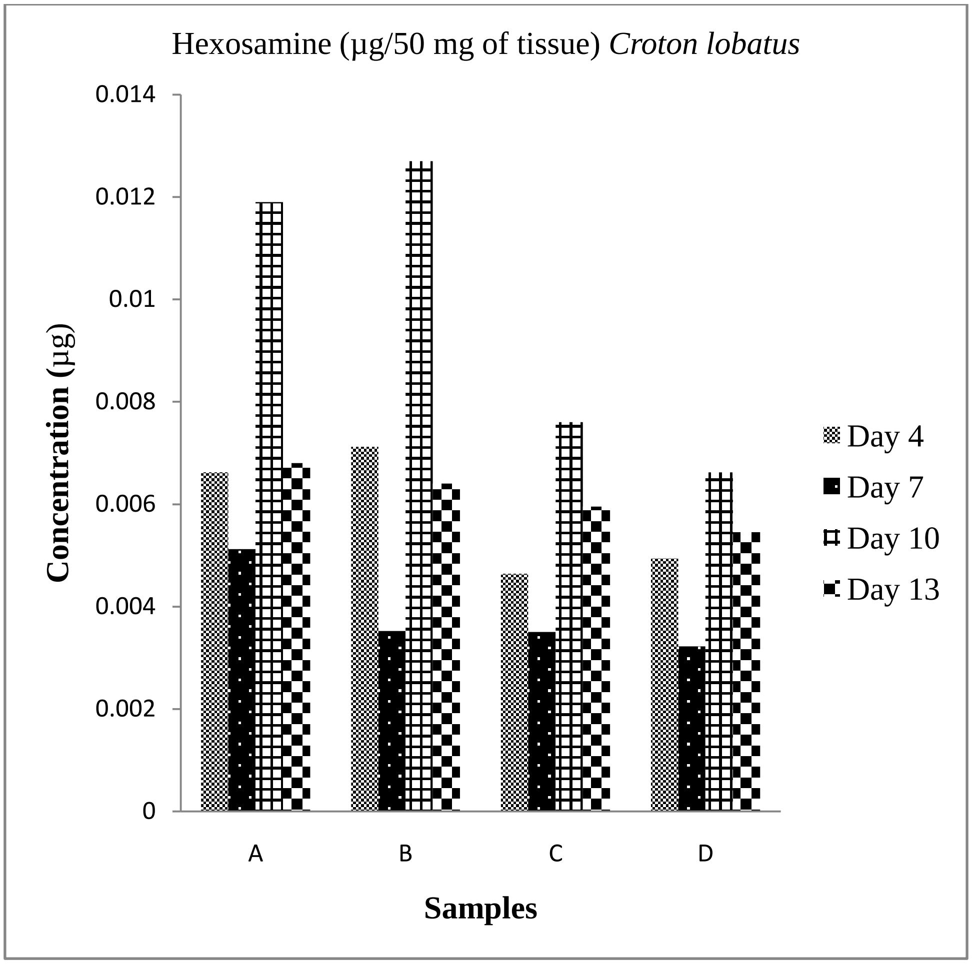

Figure 4.2a: Biochemical estimation of hexosamine (µg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (A, B, C, and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

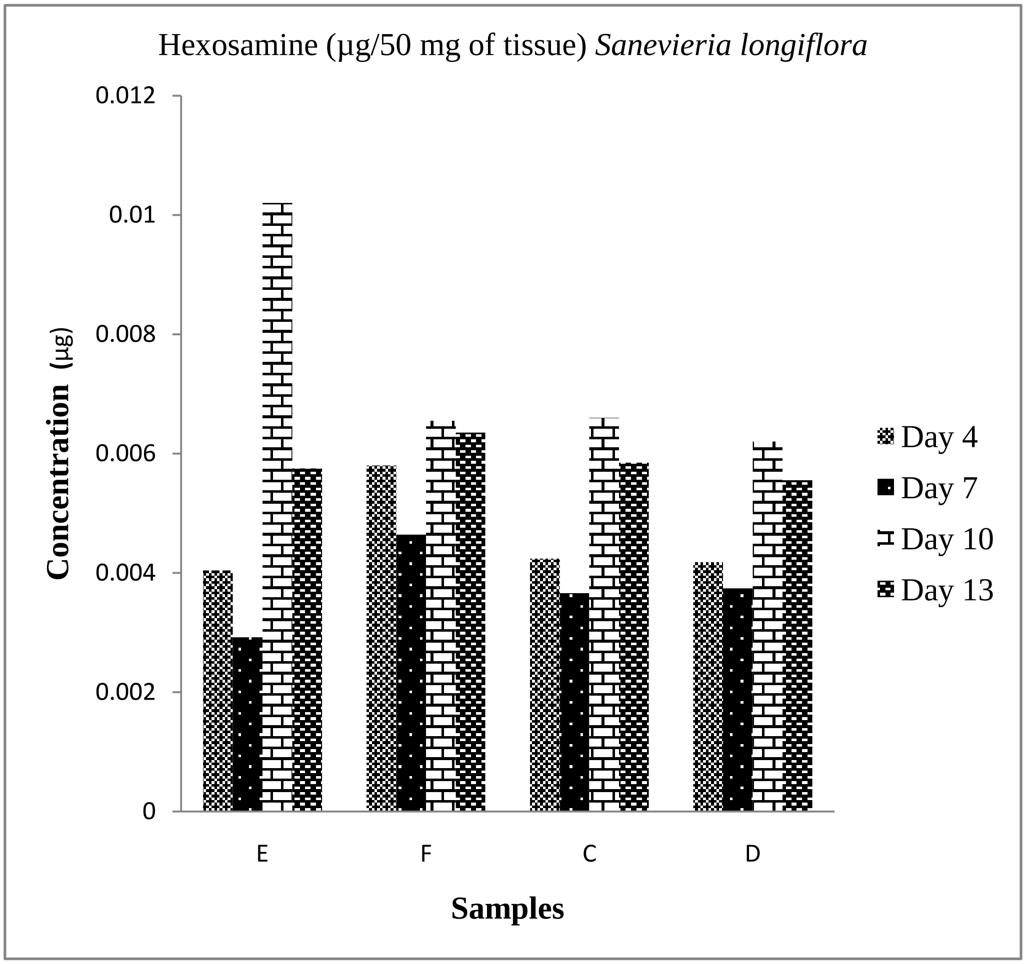

Figure 4.2b Biochemical estimation of hexosamine (µg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (E, F, C and D).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.3.3 Uronic acid

Generally uronic acid increased progressively from day 4 reaching its peak value at day 10 and then decreased in all the wounds studied (Appendix 2). *Croton lobatus* paste, *Croton lobatus* water extract, *Sansevieria longiflora* sap and *Sansevieria longiflora* water extract recorded highest trend values of uronic acid contents (Figure 4.3 a & b).

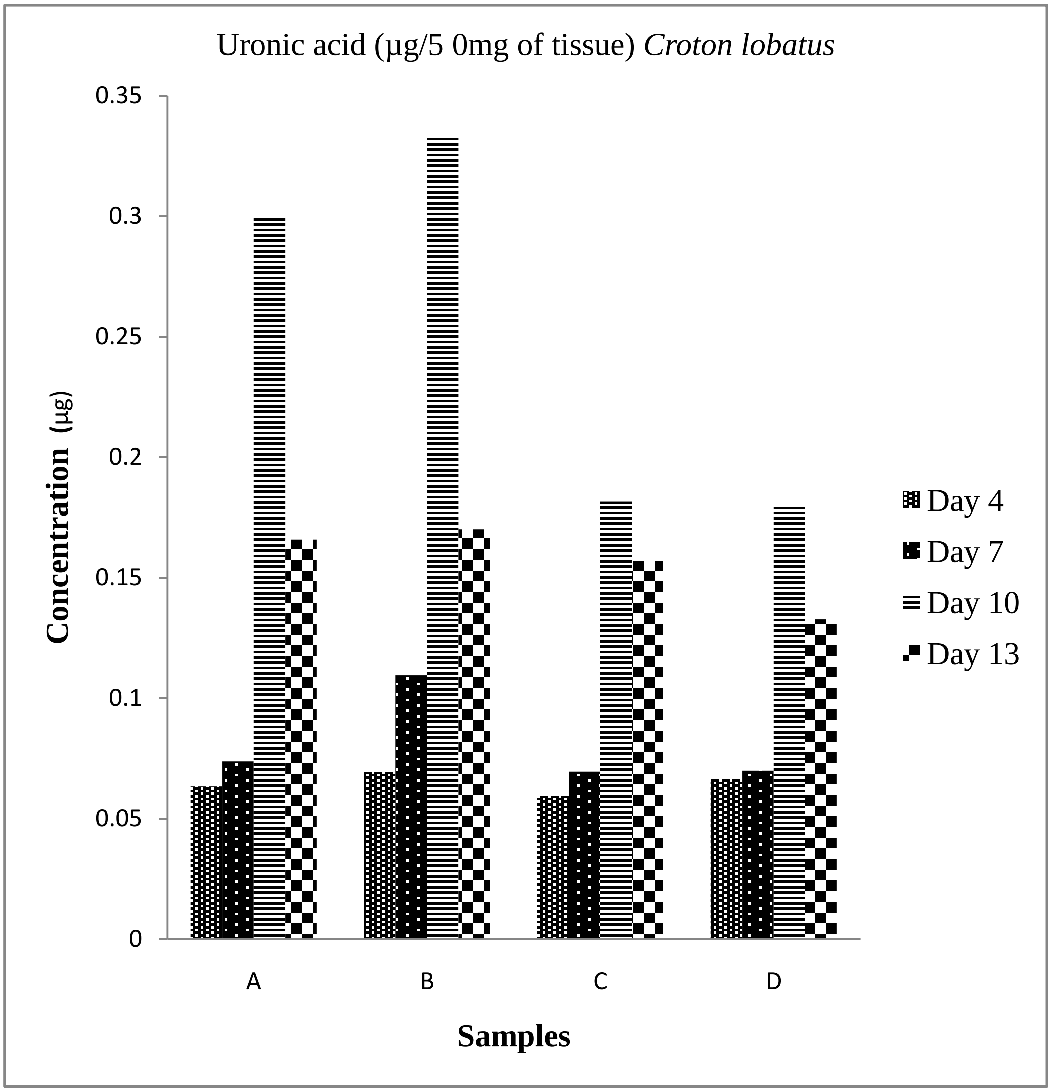

Figure 4.3a: Biochemical estimation of uronic acid (μg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (A, B, C, and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

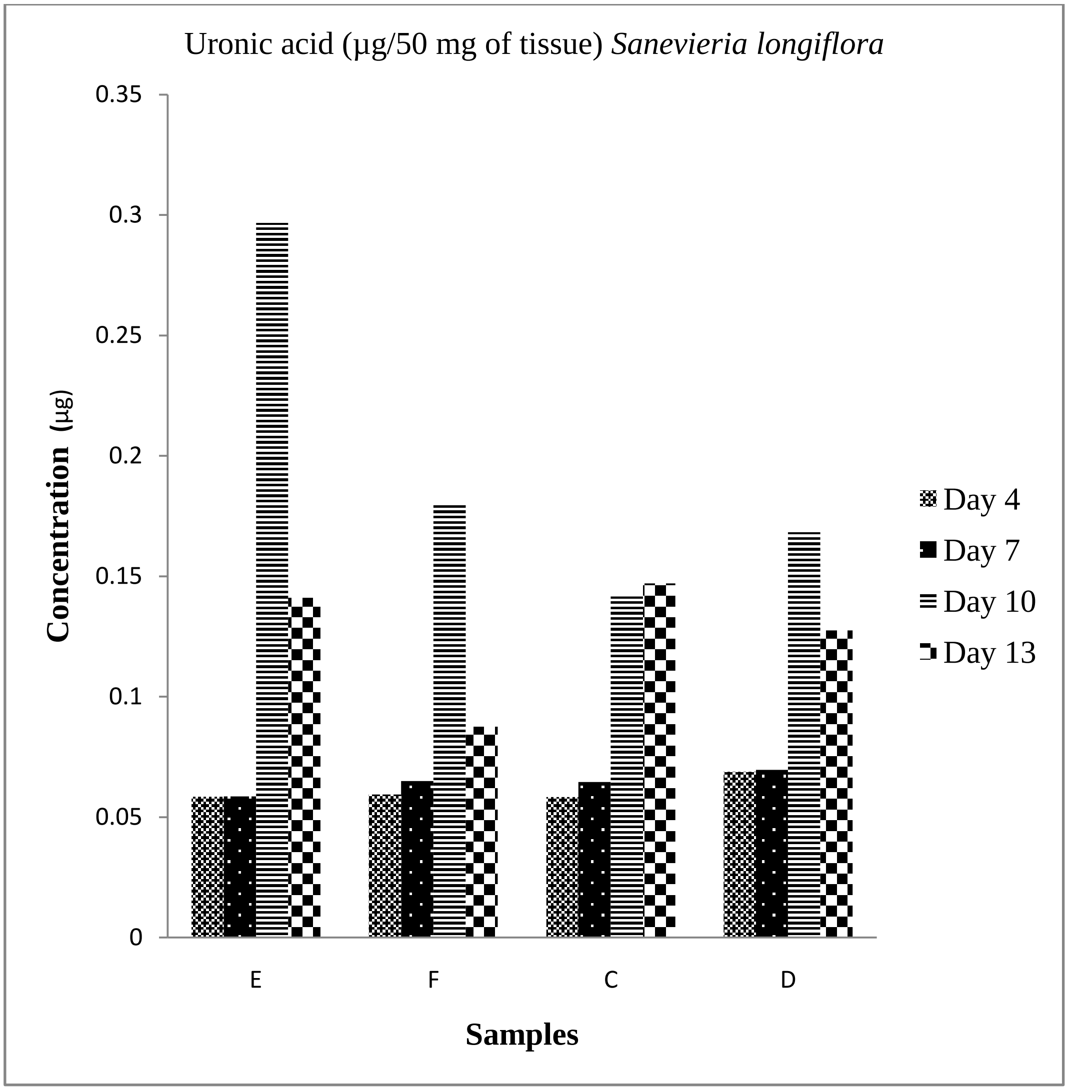

Figure 4.3b: Biochemical estimation of uronic acid (µg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (E, F, C and D).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.3.4 Total protein

The total protein content declined in value at day 7, and increased to its peak value at day 10 and then declined (Appendix 2). *Croton lobatus* paste and water extract recorded the highest value for total protein content (Figure 4.4a). For *Sansevieria* group total protein tend to decrease progressively in all the treated wounds except for *Sansevieria longiflora* sap in which it increased progressively reaching its peak value at day 10 and then declined (Figure 4.4b).

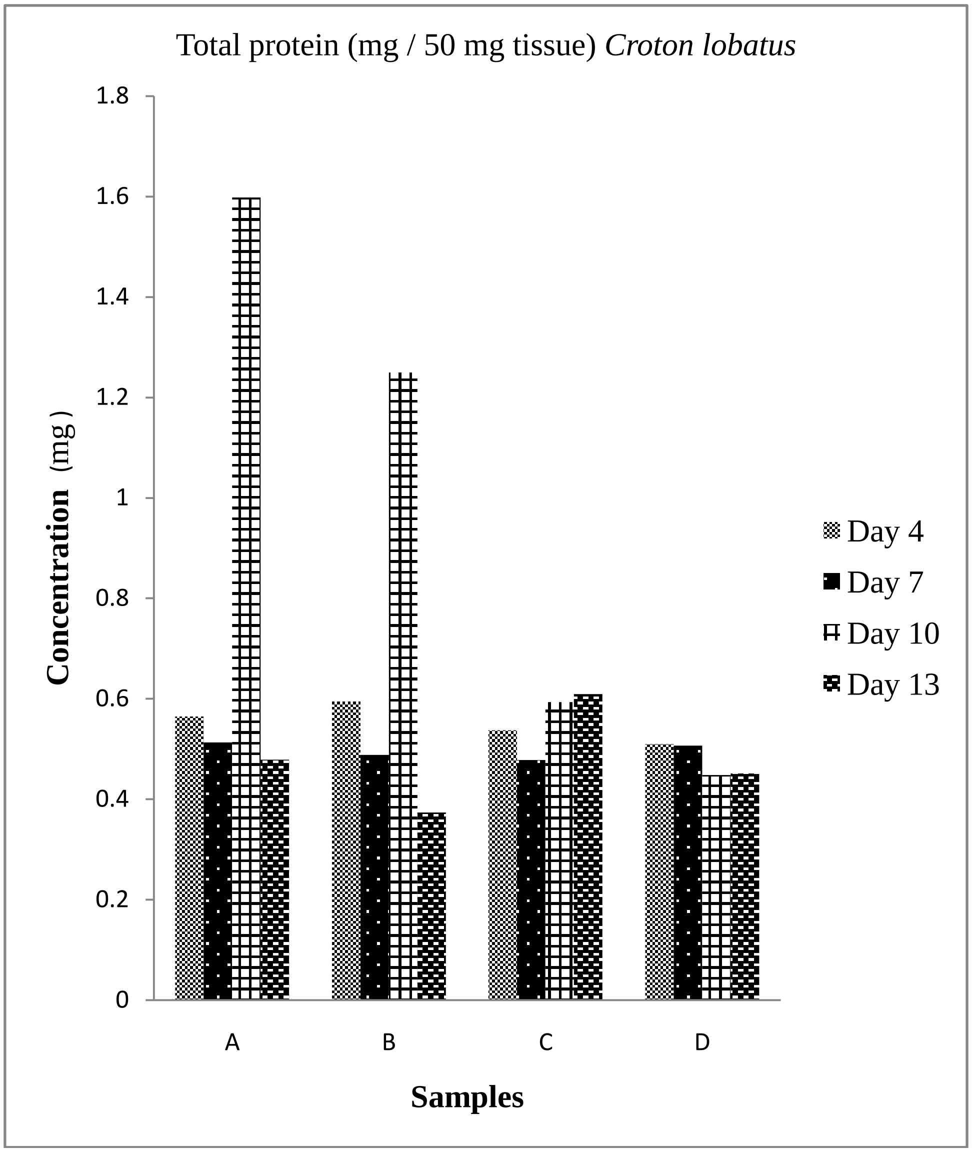

Figure 4.4a: Estimation of total protein (mg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (A, B, C, and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

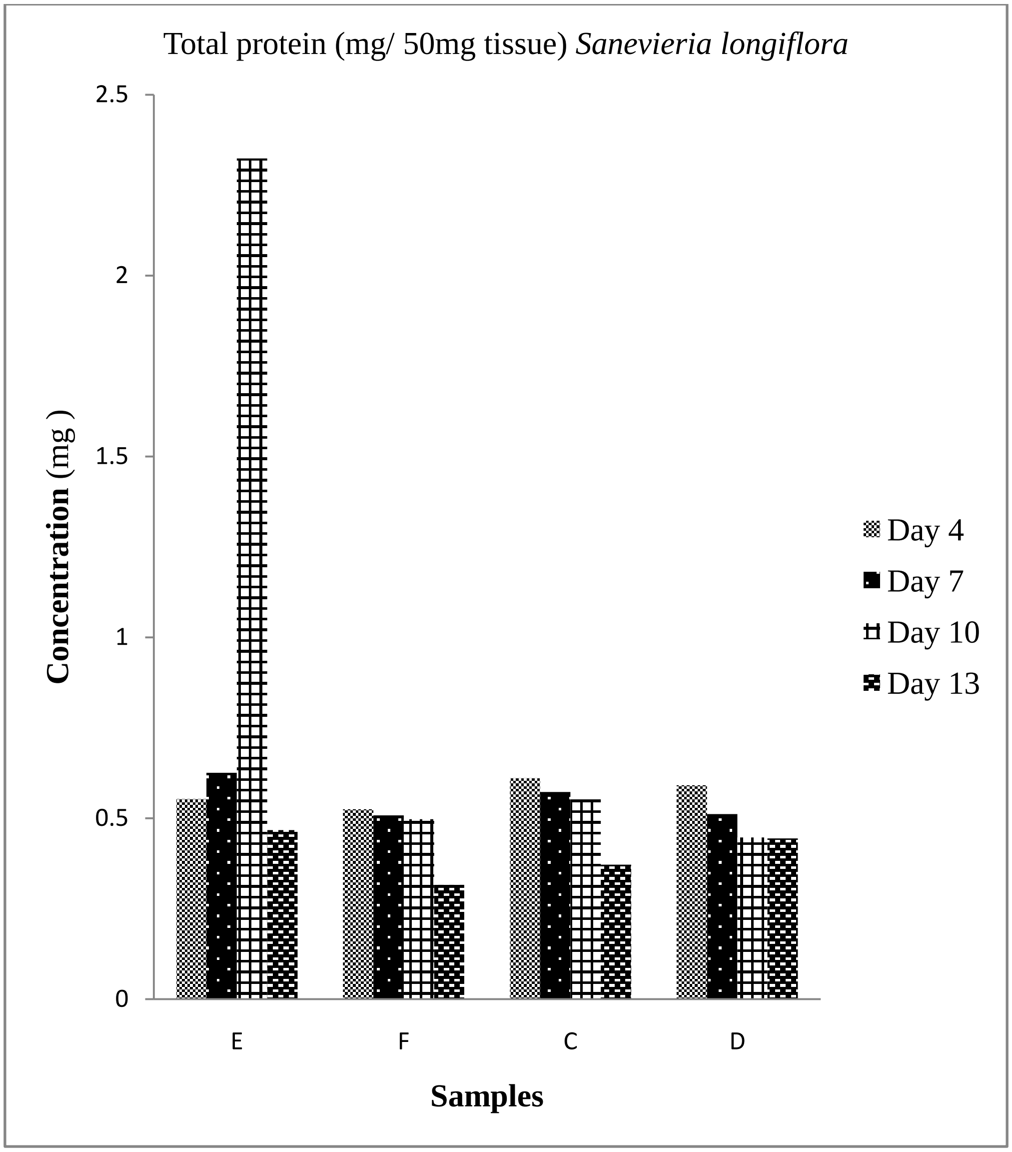

Figure 4.4b: Estimation of total protein (mg) in 50 mg of granulation tissue samples harvested from healing wounds treated with different agents (E, F, C and D)

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.4 Antimicrobial Properties of the Plant Samples

4.4.1 Antimicrobial activities of the *Croton lobatus* and *Sansevieria longiflora* extracts on wounds

4.4.1.1 Croton lobatus treated wounds: Colony forming units counted on nutrient agar reduced drastically after initiation of treatment (day 2) in all the treated wounds (A, B and D) except in physiological normal saline treated wounds (Figure 4.5a; Appendix 3c and 3d). The colony forming units counted on Mac Conkey agar significantly reduced with days on Cicatrin treated wounds, slightly reduces with days on the wounds treated with *Croton lobatus* paste while persistent high number of CFU count was recorded with physiological normal saline treated wounds and *Croton lobatus* water extract (Figure 4.5b).

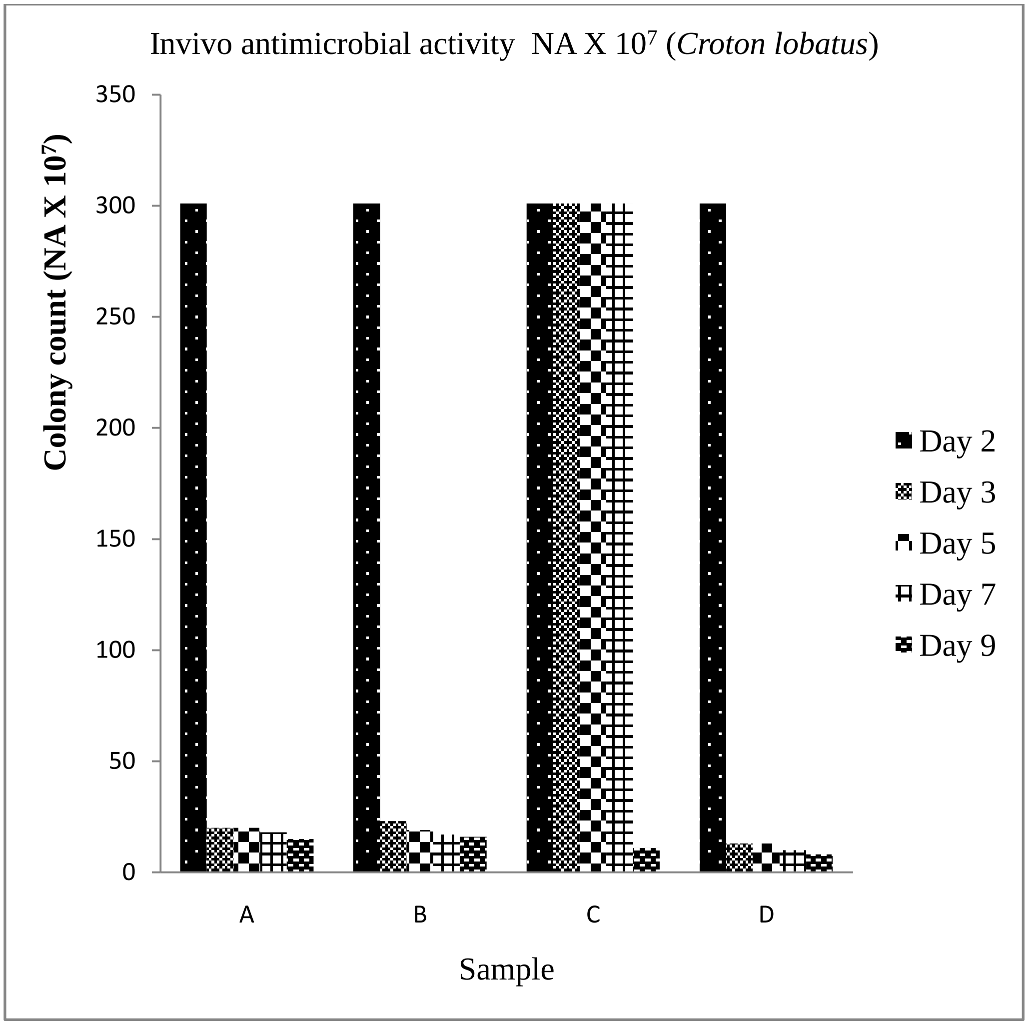

Figure 4.5a: Colony forming units (CFU) counted on nutrient agar (NA) inoculated with swaps collected from wounds treated with different agents (A, B, C and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

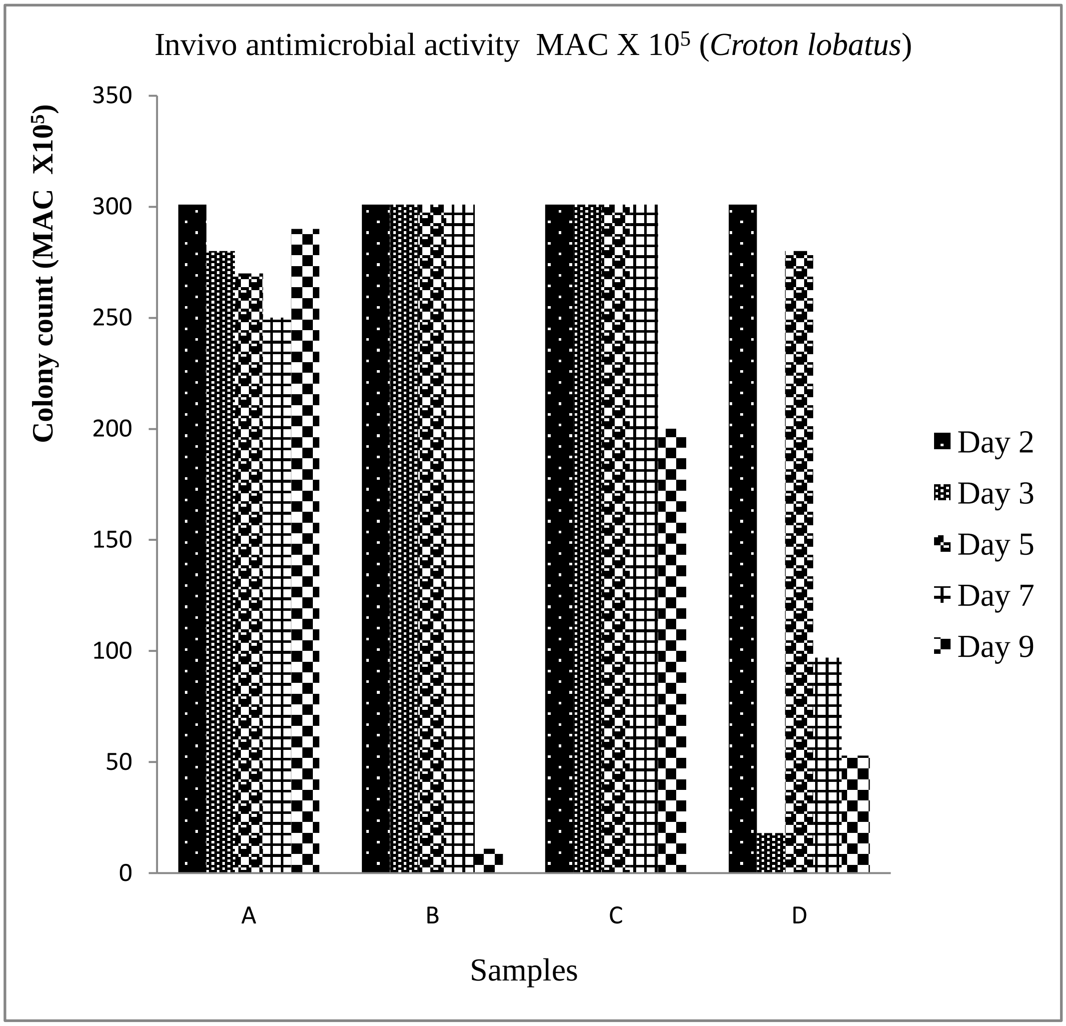

Figure 4.5b: Colony forming units (CFU) counted on Mac Conckey (MAC) agar inoculated with swaps collected from wounds treated with different agents (A, B, C and D).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.4.1.2 Sansevieria longiflora treated wounds: Colony forming units counted on nutrient media reduced significantly after initiation of treatment (day 2) in all treated wounds (A, B and D) except in physiological normal saline treated wounds (Figure 4.6a; Appendix 3e and 3f). Reduction of CFU counted on Mac Conkey agar recorded with *Sansevieria longiflora* sap was not steady unlike in Cicatrin treated wounds in which constant reduction was recorded. But with *Sansevieria longiflora* water extract and physiological normal saline treated wounds no reduction was recorded except on day 9 in which all the treated wounds showed lowest number of CFU counted.

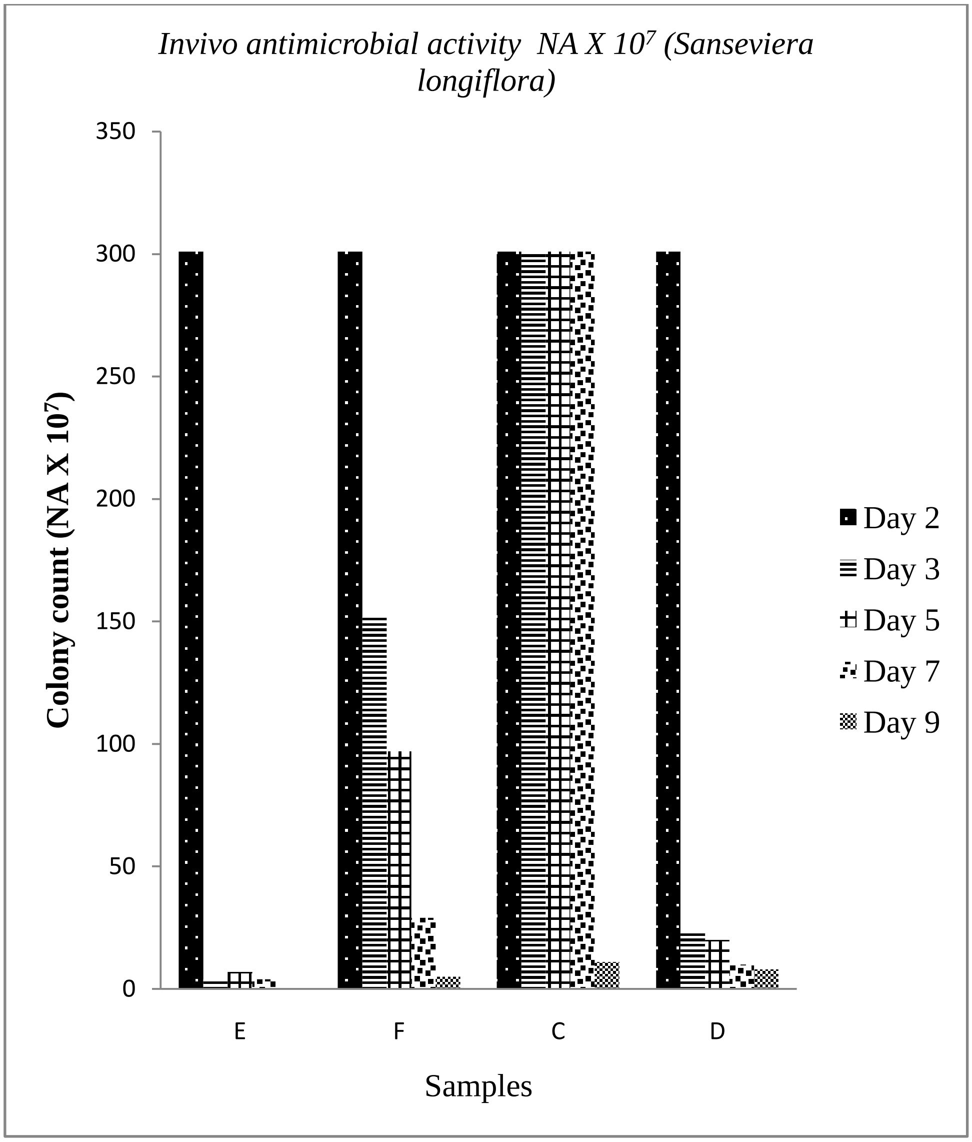

Figure 4.6a: Colony forming units (CFU) counted on nutrient agar (NA) inoculated with swaps collected from wounds treated with different agents (E, F, C and D).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

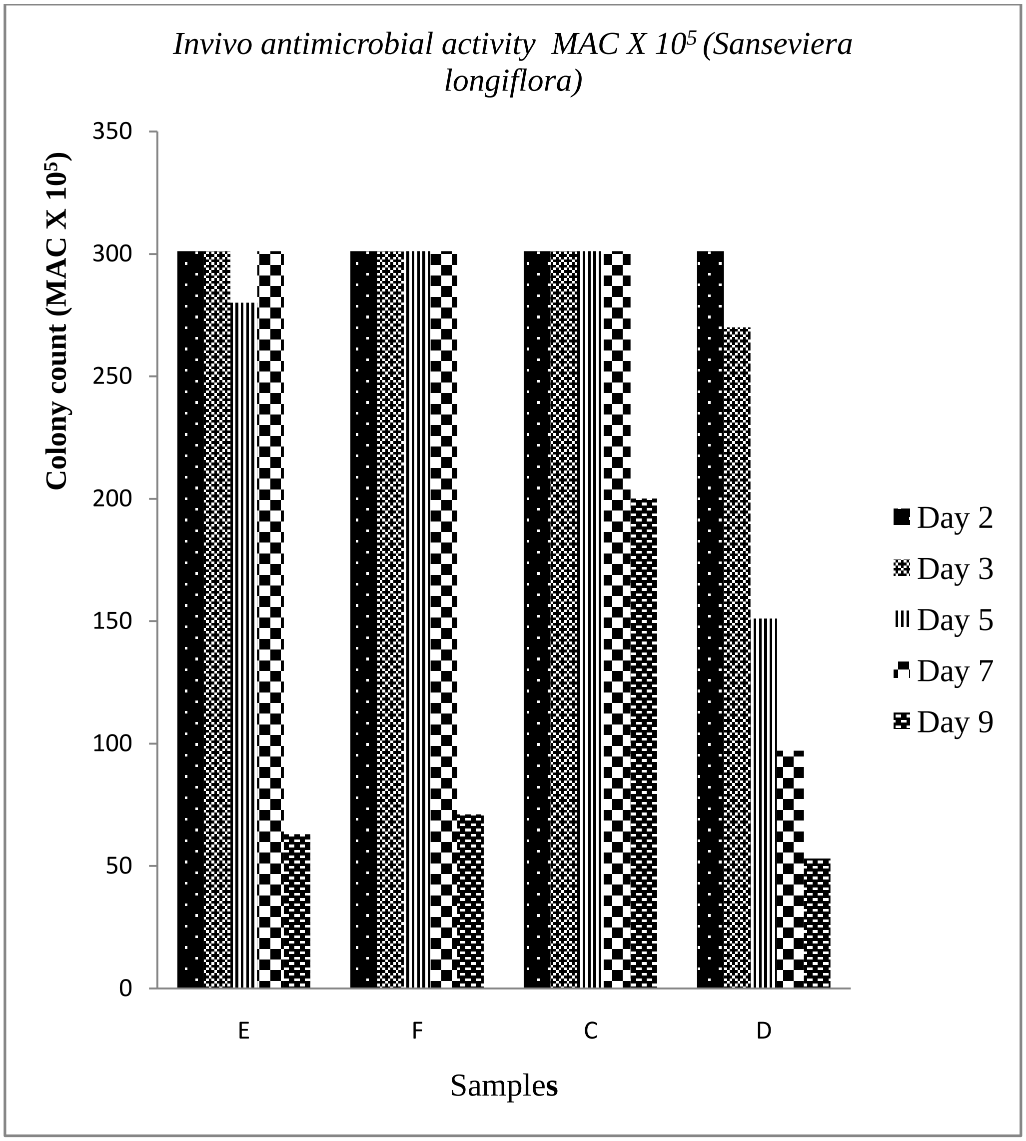

Figure 4.6b: Colony forming units (CFU) counted on Mac Conckey (MAC) agar inoculated with swaps collected from wounds treated with different agents (E, F, C and D).

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and D (Cicatrin powder).

4.4.2 *In vitro* antimicrobial studies

There was no visible zone of inhibition after 18hrs of incubation of the samples. The inoculated plates were further incubated for 24hrs after which no observable zone of inhibition from the samples. Hence, the research was repeated after one week of leaving the plant extracts in solution, this was to allow time for the active ingredients in the extract to dissolve. An agar diffusion technique with creation of wells in the agar was also employed instead of impregnating the disc with plant extracts. The observed clear zone of inhibition is represented in the Figure 4.7a and b below.

Croton lobatus paste and *Croton lobatus* 0.5 generally inhibited the growth of all the tested microorganisms (*Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*) while *Croton lobatus* 0.05 and *Croton lobatus* 0.005 reduced the growth of few organisms like Staphylococcus aureus, Streptococcus and E. coli. (Figure 4.7a; Appendix 3a). Likewise *Sansevieria longiflora* sap, *Sansevieria longiflora* 0.5 and *Sansevieria longiflora* 0.05 reduced the growth of some tested microorganisms (Figure 4.7b; Appendix 3b).

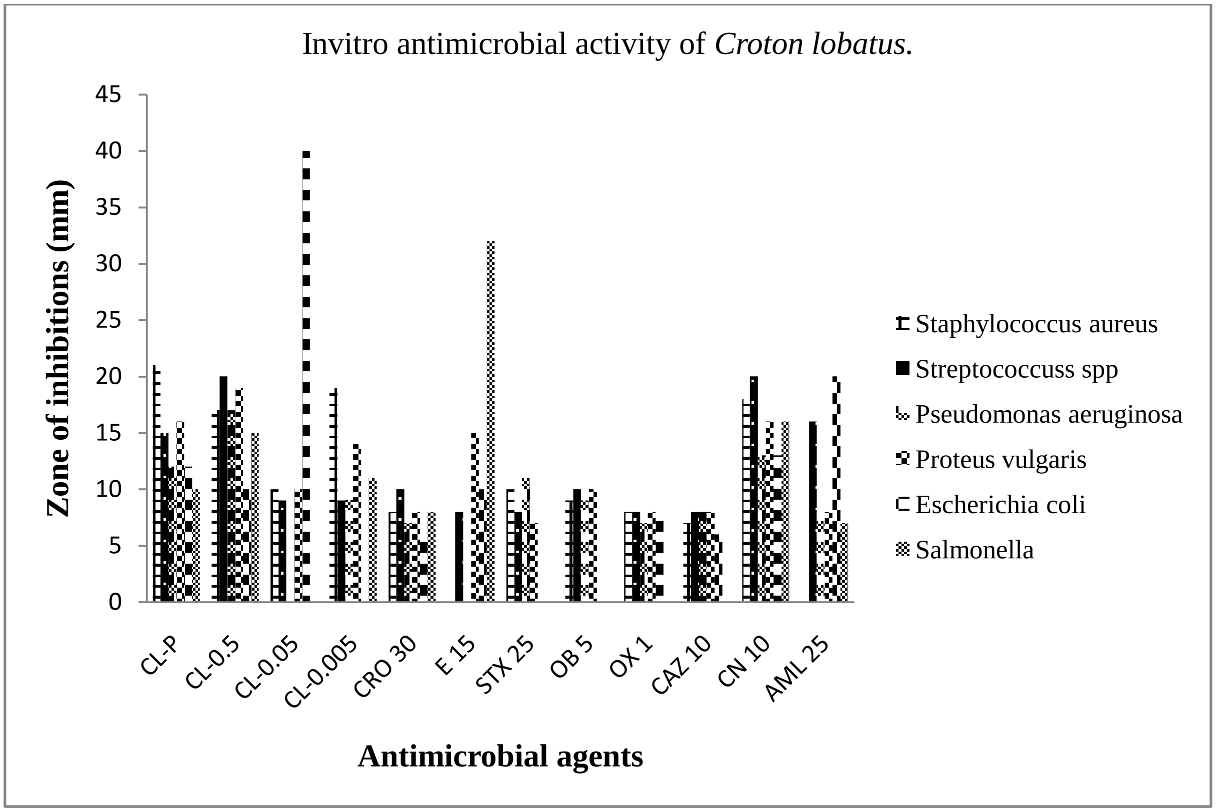

Figure 4.7a. The clear zone of inhibitions (mm) of various concentration of *Croton lobatus* plant extract compared with standard antibiotic drugs.

KEY

CL – P (*Croton lobatus* Paste), CL – 0.5 (Croton lobatus: 0.5g/ml of water), CL – 0.05 (*Croton lobatus*: 0.05g/ml of water), CL – 0.005 (*Croton lobatus*: 0.005g/ml of water), CRO 30 (Ceftriaxone), E 15 (Erythromycin), STX 25 (Sulphamethoxazole), OB 5 (Cloxacillin), OX 1 (Oxacillin), CAZ 10 (Ceftazidime), CN 10 (Gentamycin), AML 25 (Amoxycillin)

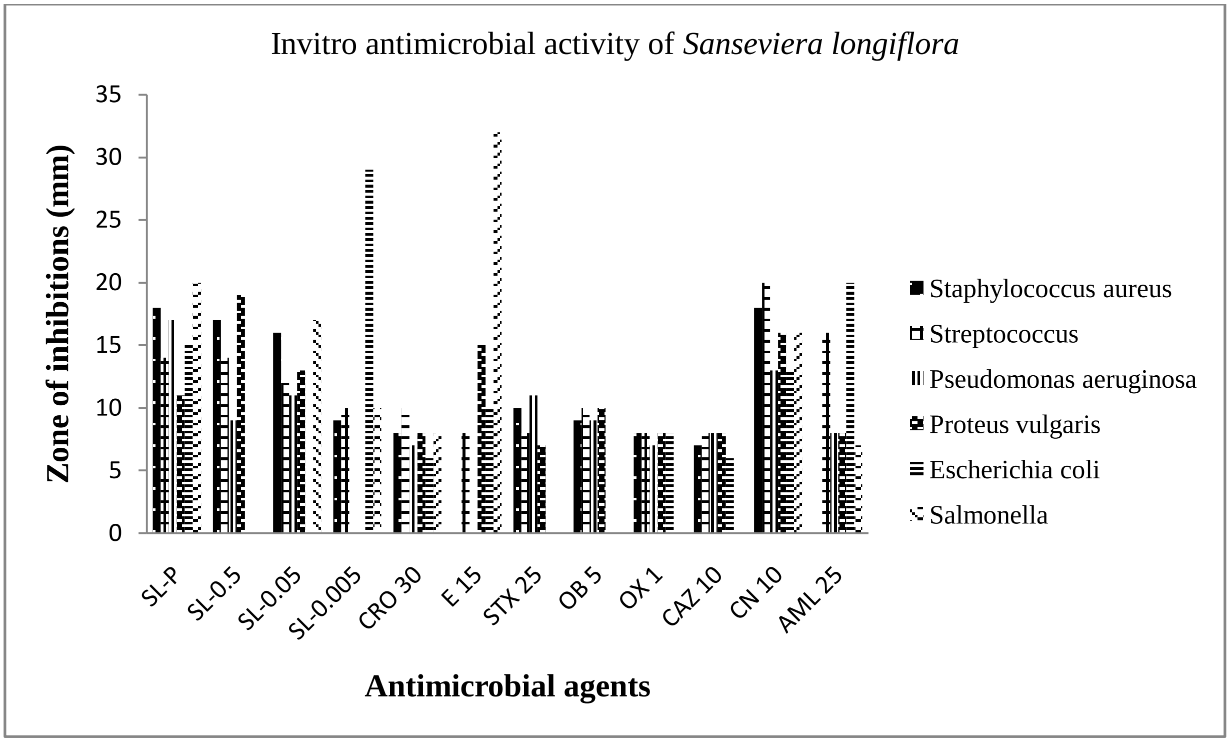

Figure 4.7b. The clear zone of inhibitions (mm) of various concentration of *Sansevieria longiflora* plant extract compared with standard antibiotic drugs.

KEY

SL – P (*Sansevieria longiflora:* Paste), SL – 0.5 (*Sansevieria longiflora:* 0.5g/ml of water), SL – 0.05 (*Sansevieria longiflora:* 0.05g/ml of water), SL – 0.005 (*Sansevieria longiflora:* 0.005g/ml of water, CRO 30 (Ceftriaxone), E 15 (Erythromycin), STX 25 (Sulphamethoxazole), OB 5 (Cloxacillin), OX 1 (Oxacillin), CAZ 10 (Ceftazidime), CN 10 (Gentamycin), AML 25 (Amoxycillin)

4.5 Analgesic Properties

4.5.1 Analgesic properties of the *Croton lobatus*

The mean values of the force (N) required in eliciting pain increased with days in all the treated groups (*Croton lobatus* paste, *Croton lobatus* water extract, *Sansevieria longiflora* sap, *Sansevieria longiflora* water extract, normal saline and xylocaine cream) with slight variation as presented in the Table 4.8 a, b and Appendix 4. Generally the normal saline group tolerated less force (more pain) while the xylocaine treated group tolerated more force (less pain) (Table 4.8a and 4.8b).

On day 1, the mean values of force required to elicit pain in *Croton lobatus* paste treated wounds was significantly higher ($p<0.05$) than the wounds treated with normal saline while in the evening the significant level increased to ($p<0.001$). On day 2, the mean values of force required to elicit pain in *Croton lobatus* water extract treated wounds was significantly higher ($p<0.05$) than the wounds treated with *Croton lobatus* paste and normal saline. While in the evening the mean value of force required to elicit pain in the normal saline treated wounds was significantly lower ($p<0.05$) than the xylocaine treated wounds. Both in the morning and evening of day 5, there is no significant difference in the forces required to elicit pain in all the treated wounds except with normal saline at ($p<0.05$) (Table 4.8a).

Table 4.8a Evaluation of analgesic property. Mean value of maximum force (N) tolerated by the animals with wounds treated using *Croton lobatus*. (n=4).

		A	B	C	G
DAY 1	Morning	5.55 ± 0.58^{a}	4.23 ± 0.38^{ab}	3.15 ± 0.26^{b}	3.93 ± 0.38^{ab}
	Evening	5.60 ± 1.44^{a}	4.2 ± 0.24^{ab}	2.85 ± 0.33^{b}	4.5 ± 0.44^{ab}
DAY 2	Morning	3.85 ± 0.29^{a}	6.6 ± 1.47^{b}	3.18 ± 0.78^{a}	5.13 ± 0.64^{ab}
	Evening	4.98 ± 1.18	4.3 ± 0.43	3.2 ± 0.22^{a}	6.68 ± 0.57^{b}
DAY 3	Morning	4.93 ± 0.37	5.65 ± 1.08	3.98 ± 0.78	6.78 ± 0.77
	Evening	4.98 ± 0.53	6.33 ± 1.54	3.85 ± 0.74	6.84 ± 0.84
DAY 4	Morning	6.18 ± 1.59	6.4 ± 0.98	6.28 ± 2.33	9.58 ± 1.26
	Evening	6.45 ± 1.23	5.73 ± 0.66	5.6 ± 1.94	7.33 ± 1.32
DAY 5	Morning	7.6 ± 0.91	9.43 ± 2.05	3.6 ± 0.47^{a}	9.73 ± 0.56
	Evening	8.50 ± 0.36	7.7 ± 1.57	3.68 ± 0.72^{a}	8.75 ± 1.34

^{ab}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$).

KEY: A (*Croton lobatus* paste); B (*Croton lobatus* water extract); C (Physiological normal saline) and G (Xylocaine cream).

4.5.2 Analgesic properties of the *Sansevieria longiflora*

On day 1, there was a significant difference in force required to elicit pain between *Sansevieria longiflora* water extract, normal saline and xylocaine cream treated wounds. On day 2, there was a significant difference ($p<0.001$) in the force required to elicit pain between the normal saline treated wounds and xylocaine treated wounds while there was no significant difference between that of normal saline and *Sansevieria longiflora* water extract treated wounds. On day 3, there was also significant different ($p<0.0001$) in force required to elicit pain between the normal saline treated wound and xylocaine cream but there was no significant difference between xylocaine cream treated wounds and that of *Sansevieria longiflora* sap. On day 4, xylocaine cream treated wounds showed significant difference ($p<0.0001$) from all other treated groups while *Sansevieria longiflora* sap was significantly ($p<0.05$) different from the normal saline treated wounds (Table 4.8b).

Table 4.8b Evaluation of analgesic property. Mean value of maximum force (N) tolerated by the animals with wounds treated using *Sansevieria longiflora*. (n=4).

		E	F	C	G
DAY 1	Morning	4.48 ± 0.57	4.2 ± 0.60	2.8 ± 0.29^{a}	5.2 ± 0.79^{b}
	Evening	3.53 ± 0.86	3.23 ± 0.36^{a}	2.58 ± 0.33^{c}	5.40 ± 0.66^{b}
DAY 2	Morning	4.3 ± 0.99	3.03 ± 0.23^{a}	2.4 ± 0.11^{a}	5.93 ± 1.36^{b}
	Evening	4.4 ± 0.65	3.08 ± 0.29^{a}	2.4 ± 0.25^{a}	5.78 ± 0.59^{b}
DAY 3	Morning	6.13 ± 0.54^{ac}	4.23 ± 0.22^{ab}	2.70 ± 0.18^{b}	7.50 ± 1.20^{c}
	Evening	5.33 ± 0.33^{a}	4.03 ± 0.71^{a}	2.70 ± 0.20^{a}	9.08 ± 1.54^{b}
DAY 4	Morning	5.60 ± 0.69^{a}	5.03 ± 0.47^{ab}	2.75 ± 0.16^{b}	9.63 ± 0.81^{c}
	Evening	4.60 ± 0.31^{a}	5.55 ± 0.51^{a}	3.95 ± 0.52^{a}	8.45 ± 1.56^{b}
DAY 5	Morning	9.83 ± 2.20	6.93 ± 0.67	5.93 ± 0.92^{a}	12.63 ± 3.78^{b}
	Evening	8.53 ± 1.19	6.93 ± 1.22	6.18 ± 0.90	9.95 ± 1.96

^{abc}Means in the same row with different superscripts alphabets were significantly different ($p<0.05$)

KEY: E (*Sansevieria longiflora* sap); F (*Sansevieria longiflora* water extract); C (Physiological normal saline) and G (Xylocaine cream).

CHAPTER FIVE

DISCUSSION

This study revealed that the components of *Croton lobatus* included: carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and tannins as similarly reported by other authors (Farnsworth, 1969; Willaman and Li, 1970; Burkill, 1985; Attioua, 2005; Chabert *et al.,* 2006). Further work was done by Barthelemy *et al.* (2012) who reported the five types of alkaloid found in *C. lobatus* aerial parts to include Palmitanoide, Onosmin B, N-(2-hydroxy-1-phenylpropyl) benzamide, Onosmin A and Aurentiamide acetate. Similar to this finding this study showed that flavonoids and alkaloids were major phytoconstituents (Table 4.1b). However, Kerharo and Bouquet (1950); Bouquet and Debray (1974) reported that the principal compound in *C. lobatus* was crotonic acid which exist in combination with toxic albuminoids. The difference in the findings was due to differences in the methods used for phytochemical analysis.

This study also revealed that *Sansevieria longiflora* contained carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and tannins as similarly present in *Sansevieria roxburghiana* (Nweze *et al.*, 2004; Philip *et al.,* 2011; Pramoda and Hanumanth, 2015), *Sansevieria trifasciata* and *Sansevieria liberica* (Ogukwe *et al.,* 2004; Sunilson *et al.,* 2009; Ikewuchi *et al.*, 2010a). However, further work on *Sansevieria roxburghiana* by Nweze *et al.* (2004); Philip *et al. (*2011); Pramoda and Hanumanth (2015) showed that it also contains quinone, proteins, Anthocyanin and betacyanin.

Tannins present in *Croton lobatus* and *Sansevieria longiflora* promote wound healing by scavenging for free radicals, anti-inflammatory and antimicrobial effects which led to healthy granulation tissues as observed during wound healing. This finding is similar to the report by several researchers (Haslam, 1996; Thakur *et al*., 2011; Akpalo *et al.,* 2015; Fakhim *et al.,* 2015; Christian *et al.,* 2016). Akpalo *et al.* (2015) observed that the process of wound healing is promoted by several herbal extracts, which are composed of active agents like triterpenes, alkaloids, flavonoids, tannins, saponins, anthraquinones, and other biomolecules. Tannins act as free radical scavengers while triterpenoids and flavonoids promote wound healing due to their astringent and antimicrobial properties (Thakur *et al*., 2011). Tannins have a wide range of pharmacological activities including antimicrobial, wound healing, antioxidant and anti-inflammatory activities (Fakhim *et al.,* 2015; Christian *et al.,* 2016). Haslam, (1996) suggested that tannins act by virtue of their complexation, astringent, antioxidant and radical scavenging activities, and their ability to form complex with proteins. Saponins appear to be responsible for wound contraction and elevated rate of epithelialisation, due to their antioxidant and antimicrobial activity (Thakur *et al*., 2011).

Flavonoids that are present in *Croton lobatus* and *Sansevieria longiflora* also aid in wound healing by serving as a potent anti-inflammatory, scavenging for free radicals in the treated wounds and improve the vascularity of granulation tissues as explained by many researchers (Geite *et al*., 2002; Thakur *et al*., 2011; Arun and Anima, 2013; Senthil *et al*., 2013; Christian *et al.,* 2016). Christian *et al.* (2016) reported that flavonoids scavenge for oxygen-derived free radicals, reduce liquid peroxidation, reduce inflammation and promote wound healing. Any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibers by increasing the strength of collagen fibers, increasing the circulation, preventing the cell damage (Geite *et al*., 2002). Flavonoids prevent or delay the onset of cell necrosis and also improve vascularity to the wounded area (Arun and Anima, 2013).

Sterols and polyphenols also reduce lipid peroxidation due to its wound healing free radical-scavenging and antioxidant activity, reducing cell necrosis and improve vascularity of the granulation tissue (Thakur *et al*., 2011). The antioxidant and anti-inflammatory activities of flavonoids were believed to be part of the important mechanisms in wound healing and in the presence of tannin it improved the regeneration and organization of the new tissue and hastened the wound healing process (Senthil *et al*., 2013).

More so, the crotonic acid which is reported to be present in the *Croton lobatus* may serve as natural ligands for perosisome proliferative activator receptors (PPAR) which are involved in wound healing and control of microbial growth as supported by other researchers. The PPAR are nuclear hormone receptors and are regulated in keratinocytes after injury and have been found to be important regulators of reepithelialisation (Goldstein *et al.,* 2003; Michalik and Wahli, 2007).

The ease of scab removal both on day 4 and 7 of wound healing in wounds treated with *Croton lobatus* paste and extract were significantly different ($p<0.0001$) than that of Cicatrin while there was no significant difference between the ease of scab removal in wounds treated with normal saline and Cicatrin powder (Table 4.2a). The ease of scab removal both on day 4 and 7 of wound healing in wounds treated with *Sansevieria longiflora* sap and extract were significantly different ($p<0.0001$) from those of Cicatrin while there was no significant difference between the ease of scab removal in wounds treated with normal saline and Cicatrin powder (Table 4.2b). There was no significant different between the amounts of fluid exudates from all the wounds treated with any of the agent (Table 4.2c and 4.2d). During debridement of Cicatrin treated wounds it was observed that a gel-like substance that was cloudy white in color, sticky and tending to draw covered the wound surface, unlike other wounds which appeared very clean with healthy granulation tissues. This made the scabs to be difficult to remove. The ease of scab removal on day 4 and 7 of wound healing in wounds treated with all the test samples (*Croton lobatus* paste, *Croton lobatus* extract, *Sansevieria longiflora* sap and extract) were significantly ($p<0.0001$) higher than that of Cicatrin while there was no significant difference between the ease of scab removal in wounds treated with normal saline and Cicatrin powder. This was due to the gel-like material that was formed which firmly attached the dead necrotic tissue to the surface of the Cicatrin treated wounds.

The highest rate of wound contraction occurred in all the treated wounds at day six because during the reparative process, fribroblasts first appear in the wound by 72 hrs after injury (Velnar *et al.*, 2009) and reach its peak after 120 hrs of injury (Alastair and Mcnaught, 2011). Once in the wound, they proliferate profusely and produce the matrix proteins hyaluronan, fibronectin, proteoglycans, type 1 and type 3 procollagen. All of their products are deposited in the local milieu (Witte and Barbul, 1997; Robson *et al.*, 2001; Ramasastry, 2005) and by the end of the first week, abundant extracellular matrix accumulates which further supports cell migration and is essential for the repair process (Sieggreen, 1987; Ramasastry, 2005). More so, this finding was also supported by Hinz (2006) who reported that wounds begin to contract about 7 days after injury, mediated mainly by myofibroblasts. According to him, interactions between actin and myosin pull the cell bodies closer together decreasing the area of tissue needed to heal. Similar to this finding, Anietie *et al.*, (2011) reported that visible wound contraction is seen only between 5 to 9 days after injury. This is however different from the finding of Eugene *et al.* (2012) who observed a sharp remarkable decrease in wound area between 1^{st} and 4^{th} day of wound healing while studying the wound healing properties of methanolic extract of dried fresh *Gossypium barbadense* leaves. The highest rate of wound contraction observed in the treatment groups were due to presence of saponins and flavonoids in the *Croton lobatus* and *Sansevieria longiflora* extracts as described by Thakur *et al*. (2011) and Geite *et al*. (2002) respectively.

At day 4 the mean percentage wound contraction in *Croton lobatus* water extract treated wounds was significantly higher ($p<0.001$) than all other treatment groups. While at day 6 the mean percentage wound contraction in *Croton lobatus* paste and *Croton lobatus* water extract treated wounds was significantly higher ($p<0.001$) than the Cicatrin treated wounds and insignificantly different from the normal saline treated wounds. This was due to increase in their collagen content as indicated by increased hydroxyproline (Figure 4.1a), increased glycosaminoglycans as indicated by increased level of uronic acid (Figure 4.3a) and increased level of protein content of the granulation tissues (Figure 4.4a). At day 4, the mean percentage wound contraction in *Sansevieria longiflora* sap and water extracts treated wounds was significantly higher ($p<0.001$) than the Cicatrin treated wounds and insignificantly different from normal saline treated wounds. While at day 6 *Sansevieria longiflora* treated wounds were significantly higher ($p<0.05$) than all other treatment groups except *Sansevieria longiflora* sap treated wounds. This was due to increase in their collagen content as indicated by increased hydroxyproline (Figure 4.1b), increased glycosaminoglycans as indicated by increased level of uronic acid (Figure 4.3b) and increased level of protein content of the granulation tissues (Figure 4.4b).

At day 10, the mean percentage wound epithelialisation in *Croton lobatus* paste and water extracts treated wounds was significantly higher ($p<0.001$) than that of Cicatrin treated wounds and insignificantly different from normal saline treated wounds, this was due to a marked increase in epithelial cell mitotic activity around the wound edges in the extract treated wound as supported by Velnar *et al.,* (2009). The plant extracts stimulated synthesis of proteins and amino acids needed by the epithelial cell mitotic activity. Similarly, the mean percentage wound epithelialisation in *Sansevieria longiflora* water extract was significantly higher ($p<0.05$) than the Cicatrin treated wound. While at day 12, the mean percentage wound epithelialisation in *Sansevieria longiflora* sap and water extract treated wounds was significantly higher ($p<0.05$) than the Cicatrin treated wounds and insignificantly different from normal saline treated wounds. This was due to a marked increase in epithelial cell mitotic activity around the wound edges in the extract treated wound as supported by Velnar *et al.,* (2009). The plant extracts stimulated synthesis of proteins and amino acids needed by the epithelial cell mitotic activity. The highest rate of wound epithelialisation observed in the treatment groups were due to presence of saponins and flavonoids in the *Croton lobatus* and *Sansevieria longiflora* extracts as described by Thakur *et al*. (2011) and Geite *et al*. (2002) respectively

This finding was similar to report by several researchers in which various medicinal plant extracts were found to have significant higher wound contraction and epithelialisation than Cicatrin treated wounds (Esimone *et al.,* 2005; Eugene *et al.,* 2012; Momoh *et al*., 2012). Eugene *et al.* (2012) observed that at day 10 after surgical creation of wounds in rats methanolic extract of dried fresh *Gossypium barbadense* leaves was able to produce about 91% healing, Cicatrin® powder produced about 80 % healing while the distilled water used as a negative control produced only about 36 % healing. Esimone *et al.* (2005) reported that Cicatrin® has very slow rate of wound healing up to the 19th day compared to *Napoleona imperialis* which showed 100 % healing at 16th day. Momoh *et al*., (2012) also observed that Cicatrin® showed a 100% healing by the 19th day after wound excision compared to the mucinated-*Occimum gratissimum* powder which showed 100 % healing by the 16th day.

Similar to the report by Thakur *et al*., 2011; Senthil *et al*., 2013; Fakhim *et al.,* 2015; Christian *et al.,* 2016, *Croton lobatus* and *Sansevieria longiflora* plant extracts scavenge for free radicals. With their antioxidant and anti-inflammatory activities they promote healthy granulation tissue formation, fibroblast proliferation and collagen synthesis thereby increasing the rate of wound contraction and epithelialisation. This is due to tannins and flavonoids that were present in *Croton lobatus* and *Sansevieria longiflora* plant extracts as described by Arun and Anima, (2013); Akpalo *et al.,* (2015); and Christian *et al.,* (2016). The crotonic acid present in the *Croton lobatus* was also reported by Goldstein *et al.,* (2003) and Michalik and Wahli, (2007). This was also supported by the observed increase in the hydroxyproline, hexosamine and total protein contents in the granulation tissues of the *Croton lobatus* and *Sansevieria longiflora* plant extracts treated wounds. Likewise, the rate of wound contraction and epithelialisation was higher in the *Croton lobatus* and *Sansevieria longiflora* plant extracts treated wounds as supported by the histological finding in which they showed abundant amount of fibroblast and collagen synthesis.

Though the mean value of the tensile strength at day 22 after the last wound healed is higher with the *Croton lobatus* paste (437.40 ± 8.79 g/cm^2) and *Sansevieria longiflora* sap

(453.60 ± 129.80); lowest with Cicatrin treated wounds (163.00 ± 27.75 g/cm^2), there was no significant difference between the mean values of all the tensile strength measurement in all the treated wounds (Table 4.6a and 4.6b). This is similar to the findings by Sieggreen, (1987) and Clark, (1993) who reported that synthesis and breakdown of collagen as well as extracellular matrix remodeling took place continuously and both tend to equilibrate to a steady state about 3 weeks after injury. Although the tensile strength of the wound increased progressively in parallel with collagen collection during the early phase (Clark, 1993; Baum and Arpey, 2005). The finding was also supported by Levenson *et al.* (1965); Broughton and Rod, (2005) who reported that all wounds gain strength at approximately the same rate during the first 14–21 days, but thereafter the curves may diverge significantly according to the tissue involved. The finding however different from the report by Prashanthi *et al.* (2012) who claimed that increase in hydroxyproline content correlates with concurrent increase in the tensile strength of the wounds (Prashanthi *et al.*, 2012). The difference was due to inability to measure the tensile strength of the harvested tissues during the early phase of the wound healing. To measure the tensile strength of healing wounds during the early phases, incisional model would have been more appropriate than the excisional approach that was used.

The mean number of days it took the wounds treated with *Croton lobatus* paste, *Croton lobatus* water extract and normal saline to heal were significantly lower ($p<0.0001$) than the wounds treated with Cicatrin as reflected by the increased hexosamine content of the wounds treated with the *Croton lobatus* past and *Croton lobatus* water extract (Figure 4.2a). Similarly, the mean number of days it took the wounds treated with *Sansevieria longiflora* sap and water extract to heal was significantly lower ($p<0.0001$) than the wounds treated with Cicatrin. Also, the time taken by the wounds treated with normal saline was significantly lower ($p<0.001$) than the Cicatrin treated wounds; this was reflected by the increased hexosamine content of the wounds treated with the *Sansevieria longiflora* sap and water extract (Figure 4.2b). The increased wound healing that was observed with the *Croton lobatus* and *Sansevieria longiflora* treated wounds was due to the presence of tannins, flavonoids, steroids and triterpenes as described by Thakur *et al*., (2011); Arun and Anima, (2013); Senthil *et al*., (2013); Akpalo *et al.*, (2015); Fakhim *et al.*, (2015); Christian *et al.*, (2016).

More so, *Croton lobatus* and *Sansevieria longiflora* treated wounds had the highest hydroxyproline content than normal saline and Cicatrin powder treated wounds. This meant that they were able to promote fibroblast proliferation thereby leading to increase in the collagen content of the wounds, hence improved wound healing. The observed increase in the hydroxyproline was due to presence of flavonoids that stimulate the collagen synthesis as described by Geite *et al*., (2002). In all the treated wounds the peak hexosamine content of the granulation tissue occurred at the day 10 of wound management, which showed that they are healing at the fastest rate as indicated by maximum rate of wound epithlialisation at day 10. However, *Croton lobatus* and *Sansevieria longiflora* treated wounds had the highest hexosamine content hence, fastest rate of wound healing. Similarly in all the treated wounds, the peak uronic acid content of the granulation tissue occur at the day 10 of wound management, which indicated an enhanced synthesis of glycosaminoglycans that was produced by fibroblast. The *Croton lobatus* and *Sansevieria longiflora* treated wounds having the highest uronic acid content meant that they were able to promote fibroblast proliferation thereby leading to improved wound healing. *Croton lobatus* paste and water extract recorded the maximum value for total protein content. This corresponds to the highest rate of epithelialisation in the treated group. Total protein in the *Sansevieria* group decreased progressively in all the treated wounds except with the *Sansevieria longiflora* sap in which it increased progressively reaching its peak value at day 10 and then declined. This explains why the highest rate of epithelialisation occurred in *Sansevieria longiflora* sap. The increased protein content of the granulation tissues harvested from the *Croton lobatus* and *Sansevieria longiflora* treated wounds was due to the presence of tannins as described by Haslam, (1996).

The *in vivo* antimicrobial studies reveals that *Croton lobatus* and *Sansevieria longiflora* treated wounds are very active against organisms that grow best in nutrient agar like *Bacillus spp, Streptococcus spp, E. coli, Pseudomonas* and *Staphylococcus spp.* So, they help in controlling wound infections thereby prevent delayed healing of wounds. The agents responsible for their antimicrobial properties are tannins, flavonoids, triterpenes and saponins as describes by Thakur *et al*., (2011); Akpalo *et al.,* (2015); Christian *et al.,* (2016). Similarly, the *in vitro* antimicrobial assay of *Croton lobatus* and *Sansevieria longiflora* plant extracts showed a dose depended activity against most of the tested microorganisms. *Croton lobatus* paste and *Croton lobatus* 0.5 generally showed activity against all the tested microorganisms (*Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*) while *Croton lobatus* 0.05 and *Croton lobatus* 0.005 showed activity against few organisms like *Staphylococcus aureus, Streptococcus* and *E. coli*. (Figure 4.7a). The antimicrobial property can be attributed to tannins, terpenoids, flavonoids, crotonic acids and saponin that is present in the leaves.

This finding is supported by several other scientists who reported the antimicrobial activities of Croton species against both Gram positive and Gram negative bacteria including *Mycobacterium smegmatis, Mycobacterium tuberculosis, Staphylococcus aureus, Bacillus subtilis,* and *E. coli* (McChesney *et al*. 1991; Peres *et al*., 1997, Abo *et al*., 1999; Salatino *et al*., 2007; Dadson *et al.*, 2012). Likewise *Sansevieria longiflora* sap, *Sansevieria longiflora* 0.5 and *Sansevieria longiflora* 0.05 showed dose dependent activities against (*Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*) (Figure 4.7b). The antimicrobial property can be attributed to tannins, terpenoids, flavonoids and saponin that is present in the leaves. This finding is also similar to the report by Yohana, (2012) and several other reports by different researchers on the antimicrobial properties of *Sansevieria roxburghiana.* Philip *et al.* (2011) reported the significant antimicrobial activity of methanol and acetone extracts of *S. roxburghiana* leaves against Gram-positive bacteria such as *Micrococcus luteus, Bacillus cereus, Enterococcus spp., Staphylococcus aureus,* Gram-negative bacteria such as *Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescence, Salmonella typhi, Salmonella paratyphi, Klebsiella pneumoniae, Shigella sonnei* and *Escherichia coli,* fungal strains *Cryptococcus spp.* and *Candida albican*. Moideen, *et al.*, (2012) reported *Sansevieria roxburghiana* root sap to possess good antibacterial activity on both gram-negative and gram-positive organisms except Streptococcus faecalis but has no substantial antifungal properties against the tested fungal strains such *as Aspergillus niger* and *Candida albicans*. While Pramoda and Hanumanth, (2015) reported significant antimicrobial activity of *S. roxburghiana* against bacteria such as *Proteus vulgaris, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Escherichia coli*.

Croton lobatus possess some analgesic properties using the force gauge tests. This was due to the flavonoids and tannins present in the leaf as described by Akpalo *et al.,* (2015); Fakhim *et al.,* (2015); Christian *et al.,* (2016). *Croton lobatus* was reported to be used as pain-killers (Burkill, 1985). In an unpublished article, Daggash also reported *Croton lobatus* to be used to assuage the pain of scorpion-sting in Nigeria. Other species of Croton have also been reportedly used traditionally as analgesic agent. The bark infusion of *C. malambo* and *C. urucurana* were used in the treatment of diabetes, diarrhea, rheumatism, gastric ulcer, anti-inflammatory, analgesic, treat wound infection and accelerate wound-healing (Peres *et al*., 1997; Peres *et al*., 1998; Orlandi-Mattos *et al*., 2002; Suárez *et al*., 2003). Similarly, *Sansevieria longiflora* possess some analgesic properties using the force gauge machine on experimental cutaneous wounds which is also due to the flavonoids and tannins present in the leaf as described by Akpalo *et al.,* (2015); Fakhim *et al.,* (2015); Christian *et al.,* (2016). This finding is similar to works done by different researchers on other species of Sansevieria. Anonymous 3, (2005) reported that *Sansevieria trifasciata is* traditionally used for the treatment of ear pain, swellings, boils and fever. Anbu *et al.* (2009) reported dose-dependent analgesic effects of ethanol and water extracts of *Sansevieria trifasciata* leaves. Jimuty *et al.* (2012) also reported the analgesic, cytotoxic and antioxidant activity of the whole plant part of *S. roxburghiana*. Other studies on *Sansevieria* species have documented its anti inflammatory activity (Mortan, 1981; Da, Silva *et al.,* 2003) analgesic property (Sunilson *et al.,* 2009).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has established the following about the use of *Croton lobatus* and *Sansevieria longiflora* in the management of wounds:

i. The phytochemicals present in both *Croton lobatus* and *Sansevieria longiflora* include carbohydrates, glycoside, saponins, steroids, triterpenes, flavonoids and tannins which were responsible for the wound healing, analgesic and antimicrobial properties.

ii. The ease of wound debridement in *Croton lobatus* and *Sansevieria longiflora* treated wounds were significantly ($p<0.0001$) higher than that of Cicatrin® treated wounds.

iii. Wound contraction in *Croton lobatus* and *Sansevieria longiflora* treated wounds were significantly higher ($p<0.001$) than the Cicatrin® treated wounds.

iv. Wound epithelialisation in *Croton lobatus* treated wounds were significantly higher ($p<0.001$) than the Cicatrin® treated wounds while the wound epithelialisation in *Sansevieria longiflora* treated wounds were also higher ($p<0.05$) than the Cicatrin® treated wounds.

v. Histopathological studies revealed that *Croton lobatus* and *Sansevieria longiflora* plant extracts promote formation of healthy granulation tissues, fewer inflammatory cell infiltration, fibroblast proliferation and collagen synthesis than the Cicatrin® treated wounds.

vi. No significant difference was observed between the mean tensile strength values of all the treated wounds (plant extracts, normal saline and Cicatrin®) at day 22 after injury

vii. Wounds treated with *Croton lobatus* and *Sansevieria longiflora* water extract healed faster than normal saline and Cicatrin® powder treated wounds and the means were 11.22 ±0.40, 12.5 ± 0.65, 13.75 ± 0.45 and 17.56 ± 1.39 days respectively.

viii. *Croton lobatus* and *Sansevieria longiflora* water extract improve wound healing by enhancing fibroblast proliferation and collagen synthesis (as indicated by high hydroxyproline and uronic acid content) and epithelialisation (as indicated by high total protein content).

ix. *Croton lobatus* and *Sansevieria longiflora* water extract inhibited the growth of common wound microbial organisms (*Staphylococcus aureus; Streptococcus Spp; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli; Salmonella Spp*).

x. *Croton lobatus* and *Sansevieria longiflora* water extract had the ability to reduce pain in healing wounds.

In conclusion, *Croton lobatus* and *Sansevieria longiflora* water extract significantly promoted wound healing.

6.2 Recommendations

i. That *Croton lobatus* and *Sansevieria longiflora* water extract be used in routine management of clinical cases.

ii. Further study should be carried out on *Croton lobatus* and *Sansevieria longiflora* using the pure isolates of the flavonoids, tannins, alkaloids, or steroids that is responsible for the wound healing, antimicrobial and analgesic properties.

iii. Further study is required to determine the toxicity and minimum dose that is required for optimal wound healing, antimicrobial and analgesic effects.

iv. Improvement on this work and commercialization of the products from these plants for human use especially in the developing countries is highly recommended.

6.3 Limitations

Unavailability of histological counting frame to establish the numerical density of fibroblast.

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Appendix 1a: Percentage wound contraction (%) recorded for each wounds from all the animals (n=18).

SAMPLE	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)	VII (%)	VIII (%)	IX (%)
DAY 4									
A	-3.13	14.58	18.52	-8.696	4.225	-3.28	0	-7.28	22.22
B	17.65	15.79	34.62	0	10.2	4.688	45	38.89	54
C	20.27	-42.86	15.22	-18.75	-3.797	18.42	29.17	38.64	28.57
D	28.38	-30	1.724	-11.67	2.941	4.167	9.677	10.81	16
E	24.24	0	18.18	16.67	31.88	15.38	53.23	21.43	47.37
F	33.33	33.33	35.14	-12.77	15.38	25	47.37	53.06	18.31
C	-1.35	16.42	15.22	-7.407	17.86	16.67	25.71	48.21	17.31
D	7.895	8	15.63	-7.143	-7.143	20.75	11.76	31.75	-1.724
DAY 6									
A	59.38	62.5	74.07	67.39	68.17	65.57	72.73	38.7	53.33
B	71.57	64.47	82.69	60	57.14	65.63	84	77.78	80
C	70.27	30	73.91	43.13	58.23	64.47	77.08	77.27	63.27
D	55.41	22.5	60.34	58.33	61.76	54.17	61.29	29.73	62
E	72.73	61.11	63.64	70.83	78.26	50	85.48	71.43	74.74
F	73.61	74.44	78.38	57.45	76.92	56.25	90.18	90.61	68.45
C	59.46	64.18	72.83	25.93	69.64	42.82	78.29	83.93	50.77
D	53.95	50	68.75	51.79	52.86	52.83	76.47	65.08	56.9
DAY 8									
A	81.25	88.54	87.04	90.87	88.73	90.16	83.64	59.77	75.56
B	94.12	89.47	86.54	84	80.61	87.81	95.5	88.89	92
C	83.78	80	84.78	81.88	82.03	86.84	92.5	90.91	91.84
D	83.78	81.25	86.21	86.67	88.24	89.58	87.74	62.16	72
E	81.82	84.72	90.45	89.72	88.41	79.49	89.68	87.14	90.18
F	90.28	94.44	90.54	77.87	84.62	79.17	94.39	95.92	85.92
C	74.32	80.6	73.91	59.26	83.93	79.49	94.29	93.57	73.08
D	71.05	78	85.94	85.71	79.29	79.25	87.65	73.97	71.72
DAY 10									
A	90.63	94.79	96.3	92.17	94.37	94.1	86.36	84.67	91.11
B	97.06	94.74	92.31	92	87.76	93.75	97	94.44	96
C	91.62	91.43	89.13	90	84.81	86.84	93.75	91.82	92.65
D	91.89	92.5	86.21	94	89.71	91.25	87.74	83.78	86.8

E	94.55	93.06	86.82	87.22	91.01	88.46	93.55	89.29	94.74
F	94.44	96.22	93.78	76.6	84.62	80.63	96.49	96.73	94.93
C	84.86	89.55	90.87	85.19	91.79	87.18	94.29	96.43	80.77
D	78.95	88.4	85.94	95.71	90	81.13	87.65	87.3	81.72
DAY 12									
A	93.75	96.88	96.3	86.96	94.37	92.46	90.91	92.34	86.67
B	97.45	94.47	93.08	93.2	89.8	90.63	97.5	95.56	95.2
C	94.59	92	86.52	93.75	89.87	94.47	93.75	40.91	92.65
D	91.62	92.5	86.21	94	93.53	91.67	88.39	88.11	84
E	93.94	93.89	93.18	94.17	92.75	91.28	96.13	91.43	92.63
F	93.89	95.56	93.24	82.98	88.46	89.58	98.25	97.14	94.37
C	94.05	91.64	93.91	93.58	91.07	86.67	94.29	96.43	80.77
D	86.84	90	86.88	92.5	91.07	90.19	89.41	92.06	82.07

Appendix 1b: Percentage wound epithelialisation (%) recorded for each wounds from all the animals (n=17)

SAMPLE	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)	VII (%)	VIII (%)	IX (%)
DAY 8									
A	70	60	51.43	52.4	67.5	56.67	94.44	42.86	63.64
B	40	50	42.86	70	55.79	48.72	88.89	60	80
C	66.67	35.71	57.14	3.45	43.66	20	88.89	80	60
D	41.67	33.33	52.5	70	25	44	89.47	0	0
E	16.67	36.36	52.38	45.9	62.5	0	77.78	28.57	
F	37.14	52	68.57	19.2	62.5	62.5	0	40	
C	36.84	53.85	25	9.09	66.67	50	0	38.57	
D	31.82	22.73	55.56	25	63.79	66.67	51.22	32.93	
DAY 10									
A	100	80	100	100	80	61.11	100	62.5	60
B	100	80	70	65	66.67	80	100	100	100
C	74.19	50	80	62.5	83.33	64	100	100	77.78
D	63.33	0	70	100	68.57	42.86	89.47	53.33	12.12
E	50	28	86.21	34.8	83.87	95	100	100	
F	60	58.82	82.61	27.3	90	90	100	77.78	
C	55.36	42.86	52.38	16.7	69.57	90	90	50	
D	37.5	46.55	73.33	16.7	64.29	90.48	0	22.64	
DAY 12									
A	100	100	100	100	100	100	100	70	100
B	100	100	100	100	68	100	100	100	100
C	85	64.29	80.65	75	82.5	100	100	100	100
D	80.65	43.33	72.5	88.9	100	100	88.89	63.64	50
E	100	100	100	61.9	100	100	100	95.24	
F	90.91	100	100	70	100	100	100	80	
C	22.73	92.86	82.14	100	92	100	90	60	
D	60	50	85.71	71.4	64	94.44	20	42.31	

Appendix 1c: Assessment of the ease of scab removal (Debridement) at day 4 and 7 during management of wounds treated with various agents.

SAMPLE	A	B	C	D	E	F	C	D
DAY 4								
Animal I	3	3	2	0	2	3	2	0
Animal II	3	3	3	0	3	3	1	0
Animal III	3	3	2	0	2	3	2	0
Animal IV	3	3	3	0	2	3	2	1
Animal V	2	3	2	0	2	3	3	0
Animal VI	3	3	1	0	3	2	1	0
Animal VII	2	3	2	1	3	3	2	0
Animal VIII	3	3	1	1	2	3	0	0
Animal IX	3	3	2	0	1	1	0	1
DAY 7								
SAMPLE	A	B	C	D	E	F	C	D
Animal I	3	3	2	1	3	2	2	0
Animal II	2	3	1	0	2	3	1	0
Animal III	3	3	2	0	2	2	2	1
Animal IV	3	3	1	0	2	2	1	1
Animal V	3	3	1	0	2	3	2	1
Animal VI	3	3	2	0	3	2	2	0
Animal VII	3	3	1	0	3	3	2	0
Animal VIII	3	3	2	0	3	3	1	0
Animal IX	3	3	2	1	1	1	1	0

SCALE:

0	Poor
1	Good
2	Very good
3	Excellent

Appendix 1d: Assessment of fluid exudates from the wounds at day 4 and 7 during management of wounds treated with various agents.

SAMPLE	A	B	C	D	E	F	C	D
DAY 4								
Animal I	3	2	3	2	2	3	3	2
Animal II	2	2	2	3	2	3	3	3
Animal III	3	3	2	3	3	2	2	3
Animal IV	3	2	2	2	2	3	2	3
Animal V	3	2	3	3	2	3	2	3
Animal VI	2	2	1	2	3	3	2	2
Animal VII	3	2	2	3	2	2	2	2
Animal VIII	3	2	3	2	3	3	3	2
Animal IX	2	2	2	2	2	3	2	2
DAY 7								
SAMPLE	A	B	C	D	E	F	C	D
Animal I	2	2	2	3	2	2	2	3
Animal II	2	2	2	2	2	3	2	2
Animal III	3	2	3	2	2	2	2	2
Animal IV	2	2	2	2	2	2	3	2
Animal V	3	2	3	3	3	2	2	2
Animal VI	2	2	2	3	2	3	2	3
Animal VII	2	2	2	2	2	2	2	3
Animal VIII	2	2	2	2	2	2	2	2
Animal IX	3	2	2	2	2	3	2	2
SCALE:								
0	None							
1	Scanty							
2	Moderate							
3	Abundant							

Appendix 1e: Wound healing time (days) for all the wounds in all the animals (n=9)

SAMPLE	A	B	C	D	E	F	C	D
Animal I	10	10	10	18	10	12	12	20
Animal II	10	10	13	19	12	13	13	18
Animal III	10	12	14	10	13	14	16	13
Animal IV	11	10	10	19	10	10	15	16
Animal V	12	12	14	21	12	12	14	15
Animal VI	12	13	13	12				
Animal VII	12	10	12	22	13	11	14	19
Animal VIII	10	12	13	16	12	12	13	14
Animal IX	12	12	12	21	14	16	13	21

Appendix 1f: Measurement of maximum load (g) that is needed to tear the healed tissue samples. That is, estimating the tensile strength of various tissue samples from various wounds at day 22 after the last wound healed.

Tag No	Sample	Length (cm)	Breadth (cm)	Breaking load (g)	Area (cm^2)	Tensile strength (g/cm^2)
1	A	1.3	0.5	290	0.65	446.15
2	B	1.4	0.7	310	0.98	316.33
3	C	2.3	1	710	2.3	308.70
4	D	2	0.9	210	1.8	116.67
5	E	1.2	0.8	560	0.96	583.33
6	F	1.8	0.6	460	1.08	425.93
7	C	1.4	0.8	290	1.12	258.92
8	D	1.7	1	230	1.7	135.29
9	A	1.4	0.6	360	0.84	428.57
10	B	1.5	0.5	340	0.75	453.33
11	C	1.4	0.7	310	0.98	316.33
12	D	1.2	0.9	320	1.08	296.30
13	E	1.5	0.7	340	1.05	323.81
14	F	1.6	0.8	330	1.28	257.81
15	C	1.4	1	270	1.4	192.86
16	D	1.9	0.8	290	1.52	190.79

Appendix 2: Concentrations of various biochemical parameters: Hydroxyproline (μg), Hexosamine (μg), Uronic acid (μg) and Total protein (mg) in 50 mg of granulation tissue samples.

Day	Sample	Total protein (mg)	Hydroxyproline (μg)	Hexosamine (μg)	Uronic acid (μg)
4	A	0.564102	0.003172	0.00662	0.06341
4	B	0.594405	0.000792	0.00712	0.06919
4	C	0.536907	0.00058	0.00464	0.05933
4	D	0.509712	0.000546	0.00494	0.06647
4	E	0.553224	0.000604	0.00404	0.05848
4	F	0.524475	0.000636	0.0058	0.05933
4	C	0.609945	0.000576	0.00424	0.05831
4	D	0.591297	0.000532	0.00418	0.06885
7	A	0.51282	0.000736	0.00512	0.07378
7	B	0.487956	0.000704	0.00352	0.10948
7	C	0.477855	0.000556	0.0035	0.06953
7	D	0.506604	0.00052	0.00322	0.06987
7	E	0.625485	0.000396	0.00292	0.05865
7	F	0.507381	0.00052	0.00464	0.06494
7	C	0.571872	0.000484	0.00366	0.0646
7	D	0.511266	0.000388	0.00374	0.0696
10	A	1.5980284	0.0014	0.0119	0.30005
10	B	1.2490275	0.00148	0.0127	0.33235
10	C	0.59317	0.00063	0.0076	0.181525
10	D	0.447552	0.00085	0.00662	0.1793
10	E	2.32323	0.00124	0.0102	0.29665
10	F	0.49728	0.00078	0.00655	0.180625
10	C	0.55167	0.00068	0.0066	0.141525
10	D	0.44702	0.00076	0.0062	0.1683
13	A	0.478632	0.00094	0.0068	0.16575
13	B	0.37296	0.0014	0.0064	0.17
13	C	0.60864998	0.000891	0.00595	0.156825
13	D	0.45066	0.00076	0.00545	0.13275
13	E	0.46717125	0.00094	0.00575	0.1411
13	F	0.315462	0.00088	0.00635	0.08755
13	C	0.371406	0.00091	0.00585	0.14705
13	D	0.443667	0.00077	0.00555	0.1275

Appendix 3a: *In vitro* antimicrobial activity of *Croton lobatus* compared with some antimicrobial drugs. The clear zone of inhibitions (mm) of various samples tested was as recorded below:

S/ N0	Agents	*Staphylococcus aureus*	*Streptococcuss spp*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	*Escherichia coli*	*Salmonella*
1	CL-P	21	15	12	16	12	10
2	CL-0.5	17	20	17	19	10	15
3	CL-0.05	10	9	0	10	40	0
4	CL-0.005	19	9	10	14	0	11
5	CRO 30	8	10	7	8	6	8
6	E 15	0	8	0	15	10	32
7	STX 25	10	8	11	7	0	0
8	OB 5	9	10	9	10	0	0
9	OX 1	8	8	7	8	8	0
10	CAZ 10	7	8	8	8	6	0
11	CN 10	18	20	13	16	13	16
12	AML 25	0	16	8	8	20	7

Appendix 3b: ***In vitro*** **antimicrobial activity of** ***Sansevieria longiflora*** **compared with some antimicrobial drugs. The clear zone of inhibitions (mm) of various samples tested was as recorded below:**

S/ N0	**Agents**	***Staphylococcus aureus***	***Streptococcus***	***Pseudomonas aeruginosa***	***Proteus vulgaris***	***Escherichia coli***	***Salmonella***
1	SL-P	18	14	17	11	15	20
2	SL-0.5	17	14	9	19	0	0
3	SL-0.05	16	12	11	13	0	17
4	SL-0.005	9	10	0	0	29	10
5	CRO 30	8	10	7	8	6	8
6	E 15	0	8	0	15	10	32
7	STX 25	10	8	11	7	0	0
8	OB 5	9	10	9	10	0	0
9	OX 1	8	8	7	8	8	0
10	CAZ 10	7	8	8	8	6	0
11	CN 10	18	20	13	16	13	16
12	AML 25	0	16	8	8	20	7

Appendix 3c: ***In vivo*** **antimicrobial activity of** ***Croton lobatus.*** **CFCs on nutrient agar media (NA X10^{7}).**

Sample	A	B	C	D
Day 2	TNTC	TNTC	TNTC	TNTC
Day 3	20	23	TNTC	13
Day 5	20	19	TNTC	13
Day 7	18	17	TNTC	10
Day 9	15	16	11	8

Appendix 3d: ***In vivo*** **antimicrobial activity of** ***Croton lobatus.*** **CFCs on Mac Conkey agar (MAC X10^{5}).**

Sample	A	B	C	D
Day 2	TNTC	TNTC	TNTC	TNTC
Day 3	280	TNTC	TNTC	18
Day 5	270	TNTC	TNTC	280
Day 7	250	TNTC	TNTC	150
Day 9	290	11	200	53

Appendix 3e: ***In vivo*** **antimicrobial activity of** ***Sansevieria longiflora*****. CFCs on nutrient agar (NA X10^{7})**

Sample	E	F	C	D
Day 2	TNTC	TNTC	TNTC	TNTC
Day 3	4	152	TNTC	23
Day 5	7	97	TNTC	20
Day 7	4	29	TNTC	10
Day 9	0	5	11	8

Appendix 3f: *In vivo* antimicrobial activity of *Sansevieria longiflora*. CFCs on Mac Conkey agar (MAC X105).

Sample	E	F	C	D
Day 2	TNTC	TNTC	TNTC	TNTC
Day 3	TNTC	TNTC	TNTC	270
Day 5	280	TNTC	TNTC	151
Day 7	TNTC	TNTC	TNTC	97
Day 9	63	71	200	41

Appendix 3g: Data of wound swaps culture from various treatment agents. Sample 1, 2 and 3 were culture from the wounds before commencement of treatment. Colony forming counts above 300 is considered too numerous to count (TNTC), + indicate presence of growth while – indicate absence of growth.

Sample No	Day	Sample I.D	MSA	EMB	SD	SSA	L.F	N.L.F	NA X107	MAC X105
1	2	none	+	+	+	-	+	-	TNTC	TNTC
2	2	none	+	-	+	+	-	+	TNTC	TNTC
3	2	none	+	+	+	+	-	+	TNTC	TNTC
4	3	A	+	+	+	+	+	-	20	280
5	3	B	+	-	-	-	-	+	23	TNTC
6	3	C	+	+	+	+	-	+	TNTC	TNTC
7	3	D	+	+	+	+	-	+	13	18
8	3	E	+	+	+	+	-	+	4	TNTC
9	3	F	+	+	+	+	-	+	152	TNTC
10	3	C	+	-	+	+	-	+	TNTC	TNTC
11	3	D	+	-	-	-	-	+	23	270
12	5	A	+	-	-	+	-	+	20	270
13	5	B	+	-	-	+	-	+	19	TNTC
14	5	C	+	-	-	+	-	+	TNTC	TNTC
15	5	D	+	-	-	-	+	-	13	280
16	5	E	-	-	+	-	+	-	7	280
17	5	F	+	-	-	+	-	+	97	TNTC
18	5	C	+	-	-	-	+	-	TNTC	TNTC
19	5	D	+	-	-	+	-	+	20	151
20	7	A	-	+	+	+	-	+	18	250
21	7	B	-	+	+	+	-	+	17	TNTC
22	7	C	+	+	+	+	-	+	TNTC	TNTC
23	7	D	+	+	+	+	-	+	10	97
24	7	E	+	+	+	+	-	+	4	TNTC
25	7	F	+	+	+	+	-	+	29	TNTC
26	9	A	+	-	+	+	-	+	15	290
27	9	B	+	-	+	+	+	-	16	11
28	9	E	-	-	-	-	-	+	0	63
29	9	F	+	-	-	-	+	-	5	71
30	9	C	+	+	+	+	+	-	11	200
31	9	D	-	-	-	-	-	+	8	53

Appendix 4: Evaluation of analgesic property. Measure of maximum force (N) that is needed in different wounds to elicit pain.

Sample	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
DAY 1	morning				evening			
Animal 1	6.6	5	2.6	3.1	9.4	4.3	2.3	4.8
Animal 2	3.9	3.2	2.8	3.5	2.4	3.5	2.7	5
Animal 3	5.9	4.3	3.6	4.3	5.3	4.5	3.8	3.2
Animal 4	5.8	4.4	3.6	4.8	5.3	4.5	2.6	5
Sample	E	F	C	D	E	F	C	D
Animal 1	5.5	4.3	3.5	4.2	5.9	3.2	3.5	5.9
Animal 2	4.4	5.3	2.9	6	3.7	4.2	2	5.7
Animal 3	2.9	2.5	2.7	3.6	2.2	2.5	2.5	3.5
Animal 4	5.1	4.7	2.1	7	2.3	3	2.3	6.5
DAY 2	morning				evening			
Sample	A	B	C	D	A	B	C	D
Animal 1	3.7	10.5	5.5	4.1	8.5	3.9	3.7	5.9
Animal 2	3.1	3.4	2.1	4.6	3.9	3.4	2.7	5.7
Animal 3	4.3	6	2.5	4.8	3.7	4.5	3.4	6.9
Animal 4	4.3	6.5	2.6	7	3.8	5.4	3	8.2
Sample	E	F	C	D	E	F	C	D
Animal 1	7.2	2.8	2.3	4.1	4.3	3	2.1	6.1
Animal 2	2.8	2.5	2.7	3.1	2.7	2.8	3.1	6
Animal 3	3.7	3.3	2.4	8.5	5.8	2.6	2	4.1
Animal 4	3.5	3.5	2.2	8	4.8	3.9	2.4	6.9
DAY 3	morning				evening			
Sample	A	B	C	D	A	B	C	D
Animal 1	5.7	6.8	5	7.3	5.9	7.2	4.3	8.9
Animal 2	5.1	7.5	5.6	7.5	5.7	6.1	5.6	5.4
Animal 3	3.9	2.6	2.4	4.5	3.6	2.3	2.1	5.3
Animal 4	5	5.7	2.9	7.8	4.7	9.7	3.4	6.3
Sample	E	F	C	D	E	F	C	D
Animal 1	5.8	3.9	3	4.9	5.7	5	2.8	4.5
Animal 2	4.9	4.7	2.5	7.3	4.5	3.8	2.1	10.1
Animal 3	7.5	3.8	3	7.1	6	2.1	3	10.5
Animal 4	6.3	4.5	2.3	10.7	5.1	5.2	2.9	11.2
DAY 4	morning				evening			

Sample	A	B	C	D	A	B	C	D
Animal 1	10.9	8.2	8.3	11.1	9.6	4.8	7.5	6.9
Animal 2	4	3.8	2	7.8	3.6	4.4	1.8	4.5
Animal 3	4.9	6	11.9	7.1	6.1	7.1	10.1	7
Animal 4	4.9	7.6	2.9	12.3	6.5	6.6	3	10.9
Sample	E	F	C	D	E	F	C	D
Animal 1	6.7	5.2	2.9	7.2	4.1	5.8	2.5	4.1
Animal 2	6.9	5	2.8	10.3	5.4	6.8	5	9.4
Animal 3	4.4	3.8	3	10.3	4.1	4.4	4.1	8.8
Animal 4	4.4	6.1	2.3	10.7	4.8	5.2	4.2	11.5
DAY 5	morning				evening			
Sample	A	B	C	D	A	B	C	D
Animal 1	4.9	15.5	4.3	8.6	9.5	11.8	4.1	5.9
Animal 2	8.8	7.2	2.6	9.2	8.5	6.9	2.3	7.1
Animal 3	8.6	6.7	4.5	11.2	8.2	4.2	5.5	10.5
Animal 4	8.1	8.3	3	9.9	7.8	7.9	2.8	11.5
Sample	E	F	C	D	E	F	C	D
Animal 1	16.1	8.6	4.1	4	11.8	9.9	4.3	4.3
Animal 2	8.4	7.2	7.5	21.5	7.2	7.9	7.9	12.9
Animal 3	5.8	5.4	7.5	9.5	6.4	4.7	7.5	10.3
Animal 4	9	6.5	4.6	15.5	8.7	5.2	5	12.3

Appendix 5: Lists of some equipments and consumables used.

Wound healing examination

Equipments: Standard surgical pack, Water bath, microtome, Glass slide, Microscope, Digital camera, tracing paper, pencil, ruler, Scissors, sample bottles, graph paper and digital force gauge machine (HF-200)

Consumables: Insulin syringe, Xylazine Hal, Ketamine Hal, gauze, chlorhexidine, Scapel blade (size 12 &23), Examination glove, Surgical glove, surgical cap and nose cover, Syringes (5ml and 10ml). Pasteur pipette, Paraffin wax, Heamatoxylene and Eosin dye, Xylene, 95 % ethanol, 10 % formaldehyde in phosphate buffered saline (PSB pH 7.0), Physiological saline solution (PSS)

Biochemical tests

Equipments: spectrophotometer, Table centrifuge, pestle and mortar, filter paper, test tube bottle, test tube bottle rag, Whatmann No 1 filter paper, pH meter, micro pipette, micro pipette tips, China dish, volumetric flask, amber bottle, electric weighing balance, glass rod, beaker

Consumables: Hydrochloric acid, Chloramine-T, Perchloric acid, P-Dimethyl aminobenzaldehyde (BDH), Ehrlich's reagent (1.33 % Dimethyl amino benzaldehyde in 1:1 ethanol: concentrated hydrochloric acid mixture), Acetylacetone, ethanolic p-methyl aminobenzaldehyde, Acetylacetone in sodium carbonate, Papain (BDH), Ethanol, Methanol, phosphate buffer, acetate buffer, Glucosamine, Bovine serum albumin (Adrich Sigma), hydroxyproline (Wuhan JCJ), glucoronolactone (Wuhan JCJ), Coomassie brilliant blue G250 (M&B; C.I 42655), Sodium tetraborate, orthophosphoric acid, distil water, L-cysteine HCl (BDH), di-sodium salt of EDTA, Sulphuric acid, and Carbazol (BDH)

Antimicrobial tests

Equipments: Micro pipette, wire loop, Bunsen burner, incubator, Consumables: Sterile swap stick, petri dish, Nutrient agar, Mac Conkey agar, mannitol sugar agar, eosin methylene blue agar, sabouraud dextrose agar, Salmonell-shigella agar, Mueller Hinton Agar (MHA), antibiotic impregnated disc (CRO 30, E 15, STX 25, OB 5, OX 1, CAZ 10, CN 10 and AML 25)

Microbial isolate: *Staphylococcus aureus* (ATCC 33591)*; Streptococcus Spp* (By Dr Raji bacteria zoonosis laboratory, A.B.U)*; Pseudomonas aeruginosa* (ATCC 9028)*; Proteus vulgaris* (isolate identified from bacteria zoonosis laboratory VPH, A.BU)*; Escherichia coli* (ATCC 43895)*; Salmonella Spp* (ATCC 4932).

Evaluation of analgesic properties

Equipments: Algometer (digital force gauge machine: HF-200), Ruler,

Consumables: Xylocaine cream,