ANTIMICROBIAL AND WOUND HEALING ACTIVITY OF *KIGELIA AFRICANA* AND *GUIERA SENEGALENESIS*

By

HAJER ELNAZEIR IBRAHIM SAEED

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Supervisor:

Professor AISHA ZOHEIR IBRAHIM ALMAGBOUL

Medicinal and Aromatic Plants Research Institute, National Center for Research , Khartoum

Co-Supervisor:

Professor NASER ELDIN BILAL, PhD

Faculty of Medical Laboratory Sciences,

University of Khartoum

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CHAPTER ONE

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المستخلص

تم اختيار الخلاصات الكلوروفورمية ، الميثانولية والمائية لنبات الغبيش(*Guiera Sengalensis*) ونبات أم مشطو ر (*Kigelia africana*) لمعرفة فعاليتها ضد خمسة أنواع من البكتريا المعيارية وهي نوعان من البكتريا موجبة الجرام (العصوية الرقيقة و العنقودية الذهبية) وثلاثة أنواع من البكتريا سالبة الجرام (الاشريكية القولونية والزائفة الزنجارية والمتقلبة الاعتياديه). ونوعين من الفطريات المعيارية (المبيضة البيضاء و الرشاشية السوداء) باستخدام طريقة الانتشار الاجاري

اثبت ان الخلاصة المائية و الميثانولية للنباتين اكثر فاعلية تجاه البكتريا و الفطريات من الخلاصة الكللورفورمية

اشتملت الدراسة على تحديد اقل تركيز مثبط لنمو البكتريا و الفطريات المعيارية لاكثر الخلاصات فاعلية ، بطريقة تخفيف الاجار.

تم تحديد فاعلية ستة مضادات حيوية مرجعية ضدالسبعة انواع من البكتريا و الفطريات المعيارية و قورنت فاعليتها مع فاعلية خلاصات النباتات المختبرة ضد البكتريا المعيارية و الفطريات المختبرة

اختيرت اكثر الخلاصات فاعلية ضد البكتريا المعيارية ومن ثم أختبرت هذه الخلاصات ضد 100 عينة بكتيرية معزولة من مرضى. تم عزلها عشوائياً بالمعمل القومي الصحي ،مستشفى الخرطوم التعليمي و مستشفى أمد رمان التعليمي .

يعتبر نبات أم مشطور أحد النباتات الطبية واسعة الانتشار في السودان و يستخدم في العديد من المستحضرات التقليدية. وقد تم في هذه الدراسة التحقق من تأثير الخلاصة الميثانولية لنبات أم مشطور على التئام جروح الجلد المفتوحة في 15 من الفئران البيضاء، السويسرية، حيث استخدمت الفئران من كلا الجنسين وبأوزان تتراوح بين80- 100)جرام حلق شعر مؤخرة الظهر والجانب الأيمن وإحداث جرح غاير دائري قطره 1 سم في المنطقة المحلوقة. وتم تحضير خلاصة ميثانولي من ثمرة النبات. كم تم أيضا تحضير مرهم 2% (وزن/وزن) من الخلاصة في ال Poly ethylene glycol .مع استخدام مرهم Fusidin كدواء قياسي ، كل من المرهمين تم مسحه مرتين يومياً

تم عمل تجربة مكونة من ثلاث مجموعات من الفئران.

قورنت المجموعات المعالجة مع المجموعات غير المعالجة حيث تم تقدير الالتئام بالنقص في منطقة الجرح. أكدت النتائج أن مرهم ثمرة أم مشطور 2% في ال Poly ethylene glycol هو عامل التئام فعال ، بل وجد انه افضل من مرهم ال Fusidin المختبر.

ABSTRACT

The chloroform, methanol and aqueous extracts of the *Guiera senegalensis* and *Kigelia africana,* were tested for their antimicrobial activity against five standard bacteria ,two standard Gram-positive bacteria (*Bacillus subtilis* NCTC 8236 and *Staphylococcus aureus* ATCC 25923), three standard Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380 and *Pseudomonas aeruginosa* ATCC 27853) and the two fungi (*Aspergillus niger* ATCC 27853 and *Candida albicans* ATCC 7596), using the cup plate agar diffusion method.

The aqueous and methanolic extracts of the two plants proved to be more effective against the bacteria and fungi tested than the chloroform extracts.

The minimum inhibitory concentrations (MICs) of the most active extracts against the standard bacteria and fungi were determined using the agar plate dilution method. The antimicrobial activity of six reference drugs were determinated against the seven tested Gram positive ,Gram negative bacteria and fungi and compared their activity with the activity of the tested plant extracts .

The most active extracts against the standard bacteria were selected, and

then they were tested against 100 clinical isolates collected randomaly from the National Health Laboratory, Khartoum Educational Hospital and Omdurman Educational Hospital.

Kigelia africana is one of the most widely used medicinal plants in Sudan, and is employed in numerous traditional preprations. In this study the wound healing effect of *Kigelia africana* fruit extract was investigated on open skin wound model on rats. 15 Swiss Wistar Albino rats of either sex weighing (80-100 gm) were used during the study Hair of the lower back and right flank of the animal was completely shaved. Full thickness circular excision wound 1 cm in diameter was made on the shaved area. Methanolic extract of *Kigelia africana* fruit was prepared. Ointment of 2% (w/w) extract in polyethylene glycol was prepared. *Fucidin* ointment was used as standard healing agent; both ointments were applied twice daily.

One trail was performed using three groups of rats. Treated groups were compared with non-treated groups.

Healing was determined by reduction in the size of wound area. The results of this study confirmed that the 2% *Kigelia africana* ointment is a potent healing agent even better than the tested Fucidin ointment.

1. Introduction and literature review

1.1 Introduction

The relationship between man and plants has always been a very close one throughout the human culture, and no doubt, the herbalist is probably one of the first professionals in the evolution of human cultures (Elghazali *et al*., 1987**).** A sizeable number of plants are used in different parts of the world for treatment of various ailments. The medicinal value of these plants was recognized since the ancient times **(**Almagboul, 1992)**.** The complexity of peculiarity of the secondary metabolism of plants makes every plant species a chemical bank of potential interest for the discovery of drugs. Since 1800, chiefly in the footsteps of traditional medicine, some 30,000 plants have been investigated according to scientific criteria for some biological action or for the presence of secondary metabolites.

The number of plant species is, however, much greater (300,000 – 500,000) and the vegetable kingdom is still and almost unexplored source of drugs, since the majority of plants have not yet been considered from the pharmacological or chemical point of view. Every living species is the outcome of a slow and irreversible process biological evolution and the ecosystems represent a precious reverse of biodiversity that has been steadily reduced by the advance of civilization **(**Michel, 1993)**.** The extinction of species through the agency of man has been documented right from preclassical times (The first known example of legal protection of a plant is probably an edict of the Assyrian kind Artaxerxes I, who in 450 BC tried to limit the felling of cedars of Lebanon, used in ship-building) and, in regard to plants, even their medicinal use has been a cause of extinction. The most well-known case is probably that of

Silphium, an umbelliferous species of the genus Ferula that grew in some areas of North Africa and whose latex was used as a spice and contraceptive (Silphium is the first documented case of oral contraception). Its commercial importance was such that the plant figured on the coins of cyrence and in the first century AD; its value was already greater than that of sivler. Several fruitless attempts were made to cultivate Silphium but in the end it becames extinct **(**Michel, 1993**).** The information contained in ancient botanical and herbal writing is usually the major source of medicinal folklore (Elghazali *et al*., 1994). The IX congress of the Italian society of pharmacognosy was held in 1998 to focus attention on modern "pharmacognosy" which is defined as the isolation and elucidation of biologically active metabolites **(**Viller *et al*., 1998). Medicinal plants continue to be of use for the treatment of disease in a world-wide basis. Plants are a logical source of new drug discovery and currently thousands are being screened for biological activities in order to develop new drug entities **(**Phillipson, 1997**)**. In recent years novel anticancer and antimalarial drugs have been developed from plant sources. Although there are many potent and specific drugs available to day for the treatment of disease, there is a public swing to alternative, complementary medicine, including the use of herbal medicine, in developed countries (Phillipson, 1997**).**

Aroma therapy is one of the most actively growing forms of alternative medicine combining massage together with counseling combining massage together with counseling and a nice odour. Most clients suffer from some kind of stress-related disease and aroma therapy encourages the healing process largely through relaxation and the relief of stress (Balchin, 1997**).**

Traditional Chinese medicine (TCM) plays an important role in health-care systems in many parts of the world **(**Ueng *et al*., 1997**).** The sale of herbal products in Europe during 1992 was 1.4 billion $ The majority of herbal products in the United Kingdom (UK) are not Licensed as medicines and are not assessed for their quality, safety and efficacy as licensed medicines. This is a matter of concern to both consumers and health-care professionals **(**Phillipson, 1997). Second world congress on medicinal and aromatic plants for human welfare was held in Buenos Aires Argentina in 1999. A total of 52 papers presented at this conference were included covering aspects including toxicity, genotoxicity and adverse effects of medicinal plants, and medicinal properties, pharmacokinetics, ethnobotany and chemical composition of medicinal plants (Martino *et al*., 1999**).** Many of the world's population can not afford medicine and rely on traditional systems of medicine which are mainly plant based. Medicinal plants require investigation in collaborative research programmes between scientists in developing countries **(**Phillipson, 1997). World Health Organization prepared a list of 20,000 medicinal plants used world wide, and indicated that 4000 drugs from plant origin are used in a wide range world wide (Omer, 2000). The demand for medicinal plants is contributing to the lost of plant species and future demand should be met from cultivated sustainable species **(**Phillipson, 1997**).** An ethnobotanical survey was conducted in 1995-1996 in the Bouhmed district of the northern part of Morocco. The use of plants by Bouhmed population for the preparation of herbal remedies has been studied. Results revealed that 96 species from 49 plant families serve for the treatment of 59 diseases (Merzouki *et al*., 1997).

Research on the ethnobotany of Mestizos in Suni Mirano in 1994 documented 60 plant species used for medicinal purposes. The scientific,

family and vernacular names, ailments treated, parts used and preparation types are tabulated for 49 species. Some cultural data on traditional healing and etiology were also collected (Jovel *et al.***,** 1996).

A new book about the healing with plants in the American and Mexican west was published in USA in 1996. The first part of this book covers the ethnohistory of this region, plant nomenclature and actions, illnesses treated with plants, and healing illnesses in women and children. In the second part there is an alphabetical list of 100 medicinal plants giving information, on nomenclature, distribution, plant description, historic, modern uses and phytochemistry (Kay, 1996). Skin care practice in Africa is undertaken under several different practices. Among the common practices is the skin care for beauty in addition to care against wounds **(**Rukangira, 2001). Although several aspects of the use of herbal remedies against psychiatric ailments in different parts of the world, including tropical West Africa, have been reviewed. There is scanty information on the application of herbal medicines in the successful treatment of mental ailments variously known in Nigeria as Ala. These include schizopherenia and other psychosomatic disorders (congenital or acquired), "normal or moon-madness and spiritual madness, believed to be caused by sorcery. The success rate of patients returning to normal family life after being treated by herbalists promoted them focus our attention on this old Nigerian medical practice on consideration of its pharmacological and economic potentials (Nwosu,1999)

Field interviews brought the total species used for disease treatment by herbalists of the majority of Baganda Tribe of southern Uganda to 168. Literature searches provided support for the ethanomedical claims for a number of these species, and provided criteria for the species

classification **(**Hamill *et al*., 2003)**.** The traditional medicine programme of the WHO defined traditional medicine as " The sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation, whether verbally or in writing" (Rukangira, 2001).

Nearly half of all prescription drugs produced in West Germany were initially derived from raw plant materials, and in USA, over 1/4 of the 500 million prescriptions dispensed annually, were derived from plants (Ayensu, 1978**).** In North Africa, plants were traditionally prescribed and used for generations and probably for centuries with slight or almost no change, and with strong belief leading in most cases to satisfactory results (Boulos, 1983).

In Africa the application of herbs for internal and external uses has always been a major factor in the practice of medicine. The treatment of wounds with decoction prepared from leaves, bark and root is a daily occurrence in an African community. Because of the astringent or disinfectant properties of certain plant parts, such applications have been highly successful for generations. The right shade, the poppy and the pea have been well known for healing qualities to the herbalists over the centuries. Modern man recognizes the familiar plant derivatives from these families as alleviants in strychnine, Quinine, Nicotine, Cocaine and morphine (Ayensu, 1978).

The relative ratios of traditional practitioners and university-trained doctors in relation to the whole population in African countries are revealing. In Ghana, for example, in Kwahu district, for every tradistional practioner, there are 224 people, compared to I trained doctor for nearly

21,000 people (Rukangria, 2001). In Sudan, medicinal folklore passed from one generation to another but was never documented. There exists however some reports (Welcome Research laboratory Reports, Sudan Notes and Records, and Brown and Massey 20). More organized institutional research and documentation on medicinal plants wer initiated by the department of Pharmacognosy-Faculty of Pharmacy. University of Khartoum.These were further developed by the establishment of the Medicinal and Aromatic Plants Research Institute in 1970, National Centre further developed these for Research, in collaboration with the department of Botany, Faculty of Science, University of Khartoum (Elghazali *etal.*, 1994). Sudan folklore-medicine represents a unique blend of indigenous cultures with Egyptian, Indi.an, Arabian, East and West African cultures (Elghazali *etal.,* 1994) The Medicinal and Aromatic plants Research Institute has drawn an urgent short-term objective to issue an atlas of medicinal plants used in Sudanese folk medicine. This in view of a number of factors such as drought, desertification, expansion of agricultural schemes and the introduction of health services to primitive areas which has initiated astonishingly rapid changes leading to the least use of native medicines which would eventually disappear (Elghazali *etal*., 1994).

In Sudan, people have been tapping their herbal remedies for medication for time immemorial. Because of this purpose they used a variety of plants ranging from the rain forest vegetation in the south to the desert vegetation of the north and from the semi-Mediterranean climatic zone of the red sea to the rich savanna of the west., The Ingassana area represents one of the richest areas in Sudan, both in medicinal plants and in the type of medication, with an interesting blend of herbal practitioners (Elghazali *etal.,* 1994).

1.1.1Rationale and objectives

1.1.2 Rationale:-

Kigelia africana and *Guiera senegalensis* are widely used by traditional healers in Sudan. Therefore this study will be carried out to provide scientific evidence for their use.

1.1.3General objectives:-

To study the antimicrobial activity of the chloroform, methanol and aqueous extracts of *Kigelia africana* and *Guiera senegalensis* against microorganisms.

1.1.4 **Specific objectives:-**

1-Testing the antimicrobial activity of plants extracts against standard microorganisms *in vitro* .

2- Susceptibility testing of the clinical isolates.

3- Determination of minimum inhibitory concentration (MIC**)** for *Kigelia africana* and *Guiera senegalensis.*

4- Wound healing activity of the most active plant extract.

1.2. Literature review

1.2.1 Antimicrobial activity of medicinal plants: -

There was a great progress achieved in the field of antimicrobial agents of plant origin during the last years (Anil *et al*., 2000).

Rode *et al.(* 1989**)** showed that a *garlic* extract has bactericidal effects against 3 of 6 Gram positive organisms tested (*Streptococcus pyogenes*, *Staphylococcus aureus, Streptococcus pneumoniae*, with minimum bactericidal concentrations (MBCs) equivalent to 3.2, 0.8 and 3.2 mg allicin / ml, respectively); and 7 of 8 Gram-negative organisms tested (*Pseudomonas aeruginosa* "2 strains", *Proteus vulgaris , Escherichia coli, Serratia marcescens, Salmonella typhimurium and Klebsiella pneumoniae*, with MBCs equivalent to 1.6 , 0.4 , 1.6 , 0.8 , 1.6 and 0.4 mg allicin/ml ,respectively).

Bandara *et al.,* (1990**)** investigated steam distillates prepared from the leaves of 10 plant species (nearly all with reported medicinal uses in the central province of Sirilanka for antimicrobial and insecticidal activity). *Murrya paniculata*, *Toddalia asiatica*, *Limonia acidissima*, *Acronychia pedunculata* and *Glycosmis penntaphylla* showed the highest antifungal activity against *Cladosporium cladssporioides*. High antibacterial activity was displayed by *L. acidissima* and *M. paniculata* against *Staphylococcus aureus*, but none of the distillates tested was active against *Escherichia coli.*

Chhabra & Uiso (1991) collected plant material of 31 species used locally for treating infections from 4 regions of Tanzania. The methanolic extracts were assayed against isolates of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Neisseria gonorrhoeae,*

*Salmonella oranienburg and Shigella boydii***.** The results were tabulated for each species; listed with its vernacular name, part used and medicinal uses. The highest antimicrobial activity was shown by *Dracaena deremensis, Acacia xanthophloea* and *Maytenus senegalensis* . Activity was more common against Gram-positive *(S.aureus*) than against Gram-negative bacteria. Of the latter pathogens, *N.gonrrhoeae* was most affected.

Almagboul, (1992**)** screened 111 Sudanese medicinal plants for their antimicrobial activity against four standard organisms **(***Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Psendomonas aeruginosa***).** Out Of the 573 extracts screened, 433 (76%) exhibited inhibitory activity against one or more of the tested bacteria.

Khan *et al*. (1993**)** investigated the antibacterial activity of *Withania coagulans* in Pakistan. The ethanol extract of the leaves and stems displayed antibacterial activity which may be due to polar components like salts, alkaloids, glycosides, saponins, polyols, resins and amino acids. The antibacterial activity exhibited by the hexane extract of stems may be due to the waxy nonpolar components of the fruit mainly fixed oil and some other minor constituents were active against some of the bacteria under investigation whereas the components of ethanol extract were active against all the micro-organisms except *S.cerevisiae*. The aqueous extract of the fruit was active and the activity might be attributed to water-soluble components.

Garg *et al. (*1994) found that various parts of neem tree have been used since ancient times in the Indian sub-continents, though the ethnobotanical knowledge is poorly documented. Based on ethnomedical reports, scientific investigations on the immunomodulatory, contraceptive

and antimicrobial activities of neem oil were undertaken. Purified extracts of neem oil (praneem) and two other active components of herbal origin were formulated as praneem herbal cream and pessaries using pharmaceutically approved ingredients. Following the completion of safety and efficacy studies, the preparations are undergoing clinical trails for contraception and in the treatment of cervicitis / vaginitis caused by various genital pathogens. Early results in these trials were very promising.

Yuh *et al*. (1995**)** extracted *Angelica pubescens* (AP) with various solvents in order to find the bioactive constituents that demonstrated analgesic and anti-inflammatory effects. The results were obtained as follows :(1) Methanol, chloroform-, and ethylacetate extracts affectively reduced the pain that was induced by 1% acetic and a hot plate. (2) Methanol, chloroform and ethylacetate extracts reduced the odema that was induced by 3% formalin or 1.5 carrageena. (3) Sixteen compounds have been isolated and identified from the roots of *Ap*. Among these compounds, columbianadin, columbiantein acetate, pergaptein, and caffeic acid significantly demonstrated antimicrobial and analgesic activities at 10mg/kg. However, only osthole and xanthotoxin revealed antimicrobial activity. Impeaorin only demonstrated an analygesic effect. These results revealed that the antimicrobial and analgesic constituents from roots of *Ap* were related to the inhibition of microbial growth and to the influence on the central nervous system.

Mahasneh *et al.* (1996**)** showed that petroleum ether, methanol, hexane, butane and aqueous crude extracts of aerial parts of *Suadeda vermiculata*, *Prosopis farcta*, *Capparis spinosa* and *Salsola villosa* exhibited antimicrobial activity against 4 bacteria (2 Gram positive and 2 Gram

negative bacteria) and 2 fungal species. The petroleum ether extract of *Suaeda vermiculata* and the butanol extract of *Salsola villosa* exhibited antifungal activity against *Candida albicans* and *Fusarium oxysporum* comparable with that exhibited by miconazole nitrate.

Valsaraj *et al*. (1997) selected from Indian traditional medicines, 78 plants on the basis of their use in the treatment of infectious diseases. Different concentrations of 80% ethanol extracts were tested, using the agar dilution method, against 4 bacteria *(Bacillus subtilis, Staphylococcus aureus, Echerichia coli and Pseudomonas aeruginosa)* and using the agar-well diffusion methods, against 2 fungi: *Candida albicans* and *Aspergillus nige*r. At the lowest tested concentration of 1.6 mg/ml, 10% of the plant extracts were active.

Ali *et al*. (1998**)** investigated ethanolic and aqueous extracts of 20 Palestinian plant species used in folk medicine for the treatment of dermatomucosal infections for their antimicrobial activities against 5 bacterial species (*Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and one *(Candida albicans*). Of the plants tested, 90% showed antimicrobial activity, with significant differences in activity between the plants. Only 10 of the tested plant extracts were active against *Candida albicans.* The ethanolic extracts (70%) showed activity against both Gram- positive and Gram-negative bacteria and 40% showed anticandidal activity, whereas 50% of the aqueous extracts showed antibactericidal and 20% showed anticandicidal activity.

Bagchi *et al*. (1999**)** found that in a survey at Lucknow, India, the seedlings of plant species which were prescribed in the Indian traditional system of medicine for a variety of infectious diseases were predominate

on fresh or decomposing cattle dung, a harsh medium for plant growth due to the high microbial load and other biotic factors, plants of most of the common species did not occur on the cattle dung heaps. It was hypothesized that plant species, which were able to grow on cattle dung, may have antimicrobial compounds in their seeds to protect them from microbial attack. In confirmation, the whole seeds of 15 of the coprophilus plant species identified as occurring most frequently on fresh decomposing cattle dung were directly tested against 8 bacterial and 3 fungal strains.

Interestingly, seeds of all the examined species exhibited antimicrobial activity. The seeds of the species found more frequently on the cattle dung heaps possessed higher levels of antimicrobial activities.

Ibrahim *et al*. (2000) investigated 70% ethanolic aerial part extracts of *Echium lonifolium*, and *Heliotropium digynum* for their biological activities at the National Research Centre, Cairo, Egypt. Different concentrations of each plant extract were used and the cork-borer method was applied to determine the antimicrobial activity. The degree of sensitivity was determined by measuring the visible and clear zone of growth Inhibition The ether extract of *Echium longifolium* was the most effective extract against the eight microorganisms used in this study. *Bacillus anthracoid* was sensitive to most of the extracts of the two plants. These plant extracts exhibited significant antimicrobial and analgesic activities.

Ramesh *et al*. (2001) found that women of the Paliyan tribes in Tirunelveli district of Tamil Nadu in India consume a bark extract of some plant species to cure menorrhagia. Aqueous and methanolic extracts and their fractions were tested against 10 human pathogenic bacteria and

4 fungal strains. Inhibitory activities were maximum in the chloroform – methanol (1: 1) fractions of the methanolic extract against *E.coli*, *K.pneumonia**e*** and *Pseudomonas aeruginosa*, which were responsible for the pathogenesis of urinary tract infection. The study provided a scientific evidence for the efficacy of their use.

Atindehou *et al*. (2002**)** tested 148 crude ethanol extracts from 115 plant species *in vitro* against Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa**)*** and the Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis*. Moreover, they were submitted to antifungal assays against *Candida albicans* and *Cladosporium cucumerinum*, a human and plant pathogenic microorganisms respectively, known to be good indicators of antifungal activity. No activity was detected against the Gram-negative bacteria while 14.8 and 10.8% of the extracts showed Gram-positive bactericidal or bacteriostatic effects on *Staphylococcus aureu**s*** and *Enterococcus.faecalis*, respectively. An antifungal activity was observed with 15 extracts (10.1%). Two species were particularly active against the fungi.

Faleiro *et al*. (2003) investigated Thymu**s** species, which is a wild species mostly, found in the arid lands of Portugal. Possible antimicrobial properties of Thymu**s** essential oils have been investigated. The chemical composition of the essential oils was analysed. The antimicrobial activity was tested by the disc agar diffusion technique against *Candida albicans*, *Escherichia coli*, *Listeria monocytogenes*, *Proteus mirabilis*, *Salmonella species* and *Staphylococcus aureus*. This study concluded that the antimicrobial activity of essential oils might be related to more than one component.

Manhal *et al.* (2004**)** tested the volatile oil, gum and resin ethanolic extracts of *Pistacia lentiscus L*. (*Misteca*), for antibacterial activity against one Gram-positive and three Gram-negative microorganisms. All extracts exhibited high antibacterial activity against the tested microorganisms. Therefore they were further tested against 14 clinical isolates. The standard bacteria were tested against two antibiotics and the results were compared with the activity of the plant extract.

Elhadi *et al. (*2005**)** showed that the oily extract of *Nigella sativa* L.seeds showed a marked antibacterial activity against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, and a promising antifungal activity against *Candida albicans*. These findings were quite similar to those reported in the literature concerning the different extracts and oil of *Nigella sativa* seeds, which were reported to possess considerable antimicrobial activities when tested against various organisms.

Mohammed *et al*. (2006) studied the antimicrobial activity of the chloroformic, methanolic and aqueous extracts of *Borreria seniensis in vitro* against five standard bacterial species*(Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) and two fungal species (*Aspergillus niger* and *Candida albicans*) by the agar diffusion method.The results indicated that the stem chloroformic extract was active against both Gram-positive and Gram-negative organisms.The stem methanolic extract showed high activity against *Bacillus subtilis*, low activity against *Escherichia coli* and no activity against *Staphylococcus aureus*,*Proteus vulgaris* and *Pseudomonas aeruginosa*.The stem aqueous extract showed high activity against both Gram-positive organisms, two Gram-negative organisms,

namely *Escherichia coli* and *Proteus vulgaris*, and was inactive against *Pseudomonas aeruginosa*.All the extracts were inactive against the two standard fungi, *Aspergillus niger* and *Candida albicans*.The active extracts were further tested against sixty clinical isolates, fifteen of each of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa*, collected randomly from specimens from Sudanese patients.

The stem chloroformic extract of *Borreria seniensis* at 200mg/ml was more effective than Ampicillin at 40µg/ml against Bacillus *subtilis* and *Proteus vulgaris*. Compared to Gentamycin 40µg/ml concentration, the extract was more effective against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The stem methanolic extract of *Borreria seniensis* at 200mg/ml was almost similar to Gentamicin 15µg/ml against *Bacillus subtilis* and Gentamycin 5µg/ml against *Escherichia coli.*

The stem aqueous extract at 200mg/ml concentration was found to be more effective than Ampicillin at 40µg/ml against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginos.* This aqueous extract was found to be similar in action to Gentamicin 15µg/ml against *Bacillus subtilis* and *Escherichia coli* and to Gentamicin at more than 40µg/ml against *Staphylococcus aureus* and *Proteus vulgaris.* The clinical isolates exhibited low susceptibility compared to the standard organisms.

Ahmed *et al.* (2007) tested the methanol and aqueous extracts of four Sudanese medicinal plants used in traditional medicine (*Acacia nilotica, Artemisia absinthium, Cyperus longus*, and *Monchma ciliatum)* for their antimicrobial activity against five standard bacteria: *Bacillus subtilis,*

Staphylococcus aureus, Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa, and three fungi: *Aspergillus flavus*, *Aspergillus niger* and *Candida albicas* using the agar diffusion method. Antimicrobial activity of these plants extracts used had antimicrobial activity against at least one of the tested standard microorganisms. Methanolic extrats were found to be more active at the five different concentrations, compared to aqueous extracts at the two different concentrations**.** *Acacia nilotica* showed the highest antimicrobial activity against the standard microorganisms compared to the other plant extracts, followed by *Artemisia absinthium, Cyperus longus* and *Monechma ciliatum* did not show a potent antimicrobial activity.

1.2.1.1 Mechanisms of actions of medicinal plants plants:

1.2.1.1.1 On microorganisms:

A review is given to various antimicrobial compounds from higher plants, covering their occurrence, chemical structures and antimicrobial properties. Like microbial antibiotics, they have specific mechanisms of antimicrobial action. Some sesquiterpenoids (e.g. in *Polygonum hydropiper*, *Warburgia stuhlmannii* and *W. ugandensis*, *Warburiga stuhlmannii* and *W.ugandensis*) damage fungal cell membranes, as do labadane-type diterpenes from *Alpinia galangal*, which also interfere with fungal lipid metabolism, other sesquiterpenoids from *Warburgia* inhibit sulfhydryl enzymes or from (*Commiphora guidottii*) cause bacterial lysis respiratory metabolism is inhibited in bacteria by diterpenes in *Podocarpus nagi* root bark, a benzoquinone derivative from *Maesa pancealate* fruits assists the antifungal action of membrane-active antibiotics. Some flavonoids (e.g. robinetin and myricetin) interfere with

DNA and RNA synthesis in bacterial cells, the unsaturated lactone porotoanemonin from *Pulsatilla alpine* acts similarly in yeasts.

Anacarolic acids from *Anacardium occidentale* assist the action of beta-Lactam antibiotics by inhibiting bacterial beta-Lactamase (Haraguchi 1998*)*.

Tegos *et al*. (2002) showed that Gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds, and multidrug resistance pumps (MDRs), which extrude toxins across this barrier. It is possible that the apparent in effectiveness of plant antimicrobials is largely due to the permeability barrier. This hypothesis was tested in study done in USA.

1.2.1.1.2 In wound healing:

Roa *et al*. (1991) found that two Indian herbs had a favourable influence on wound healing by enhancing the wound breaking strength and reduced the period of epithelization. Pieters *et al*. (1995) found that *Dragon's blood* improve wound healing *in vivo* by stimulating the formation of fibroblast and collagen.

Bodeker *et al.* (1998**)** showed in expermental evidence, that some plant extracts stimulates cell proliferation and inhibits collagen contraction, while others has antioxidant properties.

Sidhu *et al*. (1999) found that Arnebin-1 promoted cell proliferation, migration and vessel formation to form a thick granulation tissue and re-epithelization of wounds. An increase in the synthesis of collagen, fibronectin and transforming growth factor-betal was seen in arnebin-1-treated wounds compared with controls.

As transforming-betal known to enhance wound healing, and associated with wound healing defect in hydrocortisone-treated wounds, the enhanced expression of transforming growth factor-betal at both translational and transcription level by arnebin-1 may be responsible for the enhancement of wound healing during normal and impaired wound repair.

Rasik *et al.* (1999) showed that *Calotropis procera* enhance wound healing by markedly increasing collagen, DNA and protein synthesis and epithelization leading to reduction in wound area.

Austin *et al.*(2001) in Canada found that some plant species were effective in wound healing mechanism by significantly decreasing beta I integrin expression in human gingival fibroblasts that may affect cell and cell-substratum adehesion during wound healing.

Thang *et al*. (2001) showed that in cutaneous tissue repair, oxidants and antioxidants play very important roles.In local acute and chronic wounds, oxidants are known to have the ability to cause cell damage and may function as inhibitor factors to wound healing. The administration of antioxidants or free radical scavengers is reportedly helpful, notably in order to limit delayed sequelae of thermal trauma and to enhance the healing process. Extracts from the leaves of C*hromolaena odorata* have been shown to be beneficial for treatment of wounds. Studies *in vitro* of these extracts demonstrated enhanced proliferation of fibroblasts, endothelial cells and keratinocytes, stimulation of keratinocytes migration in an *in vitro* wound assay, vp-regulation of production by keratinocytes of extracellular matrix proteins and basement membrane components, and inhibition of collagen lattice contraction by fibroblasts. In this study, the anti-oxidant effects of both total ethanol and polyphenolic extracts from

the plant leaves on hydrogen peroxide and hypoxanthine-xanthine oxidase induced damage to human fibroblasts and keratinocytes were investigated.

Cell viability was monitored by a colorimetric assay. The results showed that for fibroblasts, toxicity of hydrogen peroxide or hypoxanthine- xanthine oxidase on cells was dose-dependant.

Total ethanol extract (TEE) at 400 and 800 microg/ml showed maximum and consistent protective cellular effect on oxidant toxicity at law or high doses of oxidants. Protection of cells against destruction by inflammatory mediators may be one of the ways in which the extracts from the plants, contribute to wound healing.

1.2.2 Wound healing activity of medicinal plants: -

The treatment of wound is a major reason for people seeking healthcare. Many traditional systems of medicine employ materials for this purpose but the study and use of these have been largely neglected by the medical profession in the west and by many international organizations. The relevance of research to healthcare delivary is often tenuous and must be strengthened.

There is a need to provide adequate funding for research the production of safe medicines, the training of personnel and also for the sustainable harvesting of medicinal plants. Examples to illustrate these points are given particularly from the experience of the Wound Healing Institute at Oxford, UK (Bodeker *et al*., 1998).

Pieters *et al*. (1995) evaluated the wound healing activity of *Dragon's blood*(latex from croton spp.), a traditional South American drug, and some of its constituents, including the alkaloid tapsine(applied in

polyethylene glycol(PEG)ointment or as tapsine hydrochloride in polyethylene glycol(PEG) ointment or in distilled water, the dichlorobenzufuran lignan 3,4-O-dimethylcedrusin(applied in PEG ointment or in solution in plyethylene glycol(PEG) 400) and proanthocyanidins, *in vivo* in on epidermal excision wounds in rats, and compared with the wound healing activity of synthetic drug proanthocyanidins. The beneficial effect of dragon's blood on wound healing was confirmed. *Dragon's blood* was found to be very effective in wound healing.

Adupa *et al*.(1991)showed that filtered leaf sap of medicinal plant *Tridax procumbens* increase the tensile strength of wound granulation tissue in rats with concomitant reduction in granuloma weight .This may indicate its potential in the management of keloid and hypertropic scars.

Palanichamy, (1992) assessed oinments containing a leaf extract of *Cassia* alata (a species with a wide range of medicinal uses in India and the west Indies) for wound healing effects in rabbits .The best results were obtained when the extract was formulated in polyethylene glycol base compared with bases of emulsifying wax and h-bentonit).

Nayer *et al*. (1994) used the herbal drug - Hima**x** - to treat maggot – infected- septic and lacerated wounds- foot and mouth disease and hoot lesions- and abscesses in 33 bovines. Complete cures were achieved within 7-28 days depending on the nature and severity of wound. The fly repellent nature and antimicrobial actions of himax helped in the healing process.

Ahmed *et al*. (1995) made experimental wound in 6groups of calves 5goats and 5 sheep. Two wounds were made in each animal, one wound was used as control and the other was treated topically with either *Matricaria chamomilla* {*Chamomilla recutita*} lotion or ointment; *Salix fragilis lotion*; *M.chamomilla* lotion and *Polygonum bistorta* ointment; *S. fragilis* lotion and *P.bistorta* ointment *Nigella sativa* lotion or left as control.

Clinical, histopathological, histochemical and microbial studies showed that healing was best with *M.chamomilla* lotion, followed by *M.chamomilla* lotion and *P.bistorta* oinment, *N.sativa* lotion ,*S.fragilis* lotion and *P.bistorta* oinment ,with *S.fragilis* lotion the least effective.

Dilika *et al*. (1996) found that among the Xhosa- speaking tribes in South Africa, circumcision is not just surgery, it is a cultural cermony by which men are seperated from boys.Traditionally, the wound caused by circumcision is bandaged with mashed leaves of some herbs.

As traditional circumcision has a high risk of infection, the antimicrobial properties of the plants used to bandage circumcision wounds were examined. Sterilized plant extracts were tested against the common bacteria infecting circumcision wounds (*Staphylococcus aureus, Streptococcus pyogenes*, *Streptococcus viridans* and *Escherichia coli*), using the agar plant diffusion method. These plants exihibited inhibitory effects against all tested bacteria and possessed high wound healing activities.

Kakali, (1997) investigated *L.lavandulaetolia***,** commonly known as Hallcusha, a well-known plant in Indian traditional medicine for its wound healing activity. A methnol extract of this plant (collected in West

Bengal) was examined for its wound healing activty both in the form of an ointment as well as an injection in 2 types of wound model in rats: (I) the excision wound model and (II) the incision wound model.

The injection and the ointment produced significant responses in both of the wound types tested. The results were also comparable to those of the standard drug, nitrofurazone {nitrofural} , in terms of wound contraction ability ,wound clusure time, tensile strength and regeneration of tissues at the wound site.

Bhacta, (1998) collected the leaves of *C.fistula*, used in traditional medicine in India to treat ringworm, as a purgative and for many other diseases, from Agartala,Tripura in India. The methanol extract of *C.fistula* leaves was examined for its wound healing property in the form of an ointment in two types of wound models in rats,excision wound model and incision wound model. The ointment of the leaf extract of two different concentrations (5 and 10/ w/w ointment of base) responded significantly in both models of wounds tested. The results were also comparable to that of the standard drug, nitrofurazone, in terms of wound contraction ability,epithelization period, tensile strength and regeneration of tissue at wound area.

Shukla *et al*. (1999) studied the activity of asiaticoside, isolated from *C.asiatica* in normal as well as delayed-type wound healing.

In guinea pig punch wounds (full thickness, 8mm in diameter, made using a biopsy punch), topical applications of 0.2% solution of asiacoside produced a 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epitheligation.

In streptozotocin-diabetic rat, where healing is delayed, topical application of a 0.4% solution of asiaticoside over punch wounds increased hydroxyproline content, tensile strength, collagen content. It promoted angiogensis in the chick chorioallantoic membrane model at 40μg/disk. These results indicate that asiaticoside exhibits a significant wound healing activity in normal as well as delayed healing models and is the main active constituent of *C.asiatica.*

Nagappa *et al*. (2000) found that villagers have traditionally used the poultice prepared from the fruits of *Thespesia populnea* to treat a variety of skin ailments including wounds. The aqueous extract of *T. populnea* fruit showed significant wound healing activity in the excision wound and incision wound models in rats following topical and oral administration ,respectively .

Kostava, (2001) has reviewed the literature on the chemical constituents and the biological activity of *Fraxinus ornus* bark, leaves and flowers. Chemical studies showed that the presence of many compounds belonging mainly to the groups of hydroxycoumarines, secoiridoid glucosides, phenylethanoids and flavonoids.

Biological studies reveal significant antimicrobial, antioxidative, photodynemic damage prevention, wound healing, anti-inflamatory, immunomodulatory and antiviral activities, and support the use of the folk medicine.

Martins *et al*.(2002)reported the antimicrobial and wound healing activity of the bark oil, a plant widely used by traditional healers specially for wound healing, for the first time.The essential oil was active against both bacterial and fungal strains.

Arzi *et al*. (2003) investigated *Glycyrrhiza glabra (*Licorise), one of the widely used medicinal plant employed in numerous traditional and modern preparation. The healing effect of Licorise extract was investigated on open skin wounds in rabbits. The results of this study confirmed that Licorise cream of 10 % is a potent healing agent even better than phenytoin cream.

Abdrabo *et al.* (2005) investigated the wound healing activity of *Solenostemma argel* which is one of the most widely used medicinal plants in Sudan, and is employed in numerous traditional preperations. In this study the wound healing effect of *Solenostemma argel* leaves extract was investigated on open skin wound model in rats. Thirty Swiss Wister Albino rats of either sex weighing 80-100ġ were used during the study.Hair of the lower back and right flank of animal was completely shaved. Full- thickness circular excision wound one cm in diameter was made on the shaved area.

Methanolic extract of *Solenostemma argel* leaves was prepared. Ointment of 2 %(w/w) extract in polyethylene glycol was prepared. Tetracycline ointment 3% was used as standard control; both oinments were applied twice daily.

Two trials were performed; the first using three groups of non-infected rats and the second using three groups of rats artificially infected with standardized *Staphylococcus aureus.* Treated groups were compared with non-treated groups. Healing was determined by reduction in wound area. The results of this study confirmed that the 2% *Solenostemma argel* ointment is a potent healing agent even better than the tested 3% Tetracycline ointment.

1.2.3 Antimicrobial agents: -

The year 1935 was an important one for the chemotherapy of systemic bacterial infections, although antiseptics had been applied topically to prevent the growth of microorganisms. Systemic bacterial infections had not as yet responded to any existing agents. In 1935, the red azo dye protosil was shown to protect mice against systemic streptococcal infection and to be curative in patients suffering from such infections. It was soon found that protosil was cleaved in the body to release P-aminobenzene sulfonamide, or sulfanilamide, which was subsequently shown to have antibacterial activity. These observations regarding the first sulfa drug ashered in a new era in medicine. Compounds (antibiotics) produced by microorganisms were evently discovered to inhibit the growth of microorganisms (Patric *et al*., 2005). For example, in 1928, Alexander Fleming observed that a contaminante mold was growing in a culture dish that had been carelessly left open to the air. In addition staphlococcal colonies growing adjacent to the mold were undergoing lysis. Fleming correctly concluded that the mold, later identified as strain of pencillium notatum, was producing a diffusible bacteriolytic substance capable of killing staphylococci. Fleming's unkown antibiotic, which was later named penicillin, heralded the advent of the modern antibiotic era. More than a decade passed before Fleming's discovery had practical application to the treatment of infectious disease, although injection of antimicrobial chemicals into humans was not a new concept. In 1912, Paul Ehrlich discovered his magic bullet salvarson was the first injectable substance effective *in vivo* against the spirochate of syphilis. In 1939,Florey and Chain developed a practical technique by which the antimicrobial extract of penicillium species molds could be obtained in sufficient purity and quantity for use in humans (Elmer *et al*., 1990)

Streptomycin and the tetracyclines were developed in 1940s and 1950s, follwed rapidly by development of additional aminoglycosides, semisynthetic pencillins, cephalosporins, quinolones, and other antimicrbials. All these antibacterial agents greatly increased the range of infectious diseases that could be prevented or cured (Patrick *et al*., 2005). Antimicrobial agents include naturally occurring antibiotics, synthetic derivativees of naturally occurring antibiotics (semi-synthetic antibiotics) and chemical antimirobial compouds (chemotherapeutic agents). Generally, however, the term antibiotic is used to describe antimicrobial agents (usually antibacterial) that can be used to treat infection. Compared with antibacterial agents, only a few antiviral and antifungal agents have been developed. Many antiviral agents have serious side-effects (Cheesbrough, 2004). Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable ability to develop resistance to these agents. Thus antibiotics therapy will not be the magical cure for all infections, as predicted; rather, it is only one weapon, albeit an important one, against infectious disease. It is also important to recognize that because resistance to antibiotics is often not predictable. Physicians should rely on their clincal experience for the initial selection of empirical therapy (Patrick *et al*., 2005), the need for antimicrobial susceptibility testing became evident soon after antibiotics became commercially available. Before world war, penicillin production was limited and extremely expensive. Thus, a means for predicting when the use of penciillin might cure a patient of an infectious disease was needed. During world war, additional antibiotics were discovered, and patterns of susceptibility against various organisms were established through this long- time interest in soil microbes, Waksman discovered sterptomycin in 1943, and Dubos discvered gramicidin and

tyrocidin soon thereafter. Duggar's research at Pearl River resulted in the discovery of chlortetracycline Aureomycin, by lederle laboratories (Pearl River NY) in 1944. Although these new antibiotics were truly "wounder drugs" at the time of their introduction, it was not long before resistant bacterial strains emerged. Susceptibility testing became a practical necessity. Intial optimism that antibiotics woud put an end to bacterial infection has given way to reluctant acceptance that chemotherpeutics resources must be managed wisely in order to control disease. A few bacteria such as *Streptococcus pyogenes* (Group A β –hemolytic *streptococci),* have maintained their predictable susceptibility to penicillin. This persistent susceptibility is, unfortunately, the exception rather than the rule. The mechanisms of bacterial resistant are complex, varied, and not completely understood. Lorian and colleagues have provided a detailed discussion on this complex subject. Some mechanisms are encoded by chromosomal DNA, produced by genetic mutation, and can be transferred to other bacteria by transformation or transduction. Others are mediated by extrachromosomal DNA fragments (plasmids) that can be passed from one bacterium to another and perhaps from species to anthoer, by conjugation if transfer factors are present. Even worse, some of the DNA is on transposons-genetic segments that can move between chromosomes or between chromosomes and plasmids. The major types of defects and mechanisms of resistance are summarized in Table 1. Note the multiple mechanisms of resistance may be present in a single bacterial species (Elmer *et al*., 1990). The most important concept underlying antimiccrobial therapy is selective toxicity, ie, selective inhibition of the growth of the microorganism without damage to the host. Selective toxicity achieved by exploiting the differences between the metabolism and structure of the microorganism and the

corresponding features of human cells For example, penicillins and cephalosporins are effective antibacterial agents because they prevent the synthesis of peptidoglycan, thereby inhibiting the growth of bacteria but not human cells. There are four major sites in the bacterial cell that are sufficiently different from the human cell that they serve as the basis for the action of clinically effective drugs. Cell wall, ribosomes, nucleic acids, and cell membrane, are far more antibacterial drugs than antiviral drugs. This is a consequence of the difficulty of designing a drug that will selectively inhibit viral replication. Because viruses use many of the normal cellular functions of the host in their growth .It is not easy to develop a drug that specifically inhibits viral functions and does not damagethe host cell. Broad-spectrum antibiotics are active against several types of microorganisms, eg, tetracyclines are active against many Gram-negative rods, *Chlamydiae*, *Mycoplasmas*, and *Rickettsiae,* Narrow – spectrum antibiotics are active against one or very few types.Vancomycin is primarily used against certain Gram- postive cocci , namely S*taphylococci* and *enterococci* (Warren & Ernest, 2002). In some clinical situations, it is essential to use a bactericidal drug rather than a bacteriostatic one. A bacterial drug kills bacteria whereas a bacteriostatic drug inhibits their growth but does not kill them. The salient features of the behavior of bacteriostati drugs are that: -

(1) The bacteria can grow again when the drug is withdrawn.

(2) Host defense mechanisms, such as phagocytosis, are required to kill the bacteria.

Batericidal drugs are particularly useful in certain infections, eg, those that are immediately life- threatening; those in patients below 500/ml; and endocarditis, in which phagocytosis is limted by the fibrinous network of

the regetations and bacteriostatic drugs do not effect a cure (Warren *et al*., 2002). Not all antimicrobials, at the concentration required to be effective are completely non-toxic to human cells. Most, however, show sufficient selective toxicity to be of value in the treatment of microbial disease. Antimicrobial agents can be grouped by their mode of action .i e, their ability to inhibit the synthesis of the cell membrane, cell wall, proteins, and the nucleic acids of bacteria (Cheesbrough, 2004).

1.2.3.1 Modes of action of antimicrobial agents: -

Modes of action of antibacterial & antifungal drugs are summerized in Table (2) and they include: -

1.2.3.1.1 Inhibition of cell wall synthesis:

The most important and common mechanism of antibiotic activity is interference with baterial cell wall synthesis. Most of the cell wall –active antibiotics are classified as β –lactam antibiotics (e.g, penicillins, cephalosporins, cephamycins, carbapenems, monobactams, β=lactamase inhibitors), so named because they share a common β-lactam ring structure, other antibiotics that interfere with construction of the bacterial cell wall include vancomycin, bacitracin, and the following antimycobaterial agents : isoniazid, ethambutol , cycloserine, and ethionamide.

β-lactam antibiotics: - The major structural component of bacterial cell wall is the peptidoglycan layer. The basic structure is a chain of 10 to 65-disaccharide residue consisting of alternating molecules of N-acetylglocosmine and N- acetylmuramic acid. These chains are cross-linked with peptide bridges that create a rigid mesh coating for the bacteria.

(1) Penicillin antibiotics are highly effective antibiotics with an extremely low toxicity. The basic compound is an organic acid with a β-lactam ring obtained from culture of mold penicillium chrysogenum.

(2) Cephalosporins and cephamycins: - The cephalosporins are β- lactam antibiotics derived from 7aminocephalosporanic acid (the B-lactam ring is fused with a dihydrothiazine ring) that was originaly isolated from the mold cephalosporium.

(3) Other B-lactam antibiotics.

(4) Glycopeptides: Vancomycin.

(5) Polypeptides: Bacitracin and polymyxins.

(6)Isoniazid, ethionamide, Ethambutol and cycloserine (Patrick *et al*., 2005).

1.2.3.2 Inhibition of protein synthesis: - Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein syntesis in human cells. This selectivity is due to the differences between bacterial and human ribosomal proteins, RNAs, and, associated enzymes. Bacteria have 70s ribosomes with 50s and 30s subunits, whereas human cells have 80s ribosomes with 60s and 40s subunits. Chloramphenicol, erythromycin, clindamycin, and linezolid act on the 50s subunit, whereas tetracyclines and aminoglycosides act on the30s subunit. A summary of the modes of action of these drugs is presented in Table (3).

1- Drugs that act on the 30s subunit.

Aminoglycosides: Aminoglycosides are bacterial drugs especially useful against many Gram-negative rods. Certain aminoglycosides are used

against other organisms; eg, streptomycins used in multidrug thearpy of tuberculosis.

Tetracyclines: tetracyclines are a family of antibiotics with bacteriostatic activity against a variety of Gram- postive and Gram –negative bacteria.

2- Drugs that act on the 50s subunit: -

Chloramphenicol: chloramphenicol is active against a broad range of organisms' inculding Gram negative and Gram postive bacteria.

Erythromycin: erythromycin is a bacterostatic drug with a wide spectrum of activity.

Clindamycin: the most useful clinical activity of this bacterostatic drug is against anaerobes, both Gram postive and Gram negative bacteria (Warren and Ernest 2002).

1.2.3.3 Inhibition of nucleic acid synthesis: -

Quinolones: The quinolones are one of the most widely used classes of antibiotics.

These are synthetic chemotherapeutic agents that inhibit bacterial DNA topoisomerase type ii (gyrase) or topoisomerase type IV, which are required for DNA replication, recombination, and repair.

Rifampin and Rifabutin: Rifampin, a semisynthetic derivative of rifampcin B produced by Streptomyces mediterranei –binds to DNA dependent RNA polymerase and inhibits the intiation of RNA synthesis.

Metronidazole: metronidazole was originally introduced as an oral agent for the treatment of Trichomonas vaginitis. However, it was also found to be effective in the treatment of amoebiasis (Patrick *et al*., 2005),

Antimetabolites: The sulfanomides are antimetabolites that compete with P- aminobenzoic acid therepy by preventing the synthesis of the folic acid required by certain microorganism.Because mammalian organisms don't synthesize folic acid (required as a vitamin). Trimethoprim is other antimetabolites that interfere with folic acid metabolism by inhibiting dihydrofolate to tetrahydrofolate (Patrick *et al.*, 2005).

1.2.3.4 Additional drugs mechanisms: -

-. Isonizid: inhibits mycolic acid synthesis.

Metronidazole (flagyl): This drug has two possible mechanisms of action; the first one is its ability to act as electron sink. The second mode of action of metronidazole is related to its ability to inhibit DNA synthesis by unknown mechanism (Warren and Ernest 2002).

1.2.4 Antimicrobial susceptibility testing: -

The use of *in vitro* susceptibility testing in clinical laboratories is an attempt to predict the likely *in vivo* response of the infecting organism to selected range of antimicrobial agents. Such tests are carried out very widely, but their limitations need to be appreciated in that organisms are tested under conditions favouring rapid growth on highly nutritional media and no account is taken of factors outside the organism – antibiotic interaction. Susceptibility tests are designed to give a result interpreted as susceptible, intermediate or resistant (S, I or R). A patient infected with a susceptible organism should respond to the manufacturer's recommended dosage regimen, whereas one infected with a resistant organism would be unlikely to respond. For an organism categorized as intermediate (or moderately susceptible), there is uncertainity whether or not the patient

will respond to standard doses, but he or she will be more likely to respond to higher doses or if concentrations in excess of those in the plasma are obtained at the site of infection. However, it is term which clinicans generally find unhelpful (Hawkey and Lewis, 1989). The primary role of the clinical microbiology laboratory is to provide information with which physicians can diagnose and treat infectious diseases. If a communicable disease is present, the identification of a specific pathogen is of ulmost important to a hospital epidemiologist or puplic health worker. Identification of a microbe has been recovered from a clinical specimen often benefits the patient by definitively identifying apuzzling disease and assisting in the provisional selection of chemotherapy, but the two most important pieces of information for clinicians are: -

(1) Whether an infectious agent is present and

(2) Which antimicrobial agent should provide adequate therapy? These priorities were derived from one of the great medical advances of this century (Elmer *et al*., 1990). In the treatment and control of infectious diseases, especially when caused by pathogens that are often drug resistant, susceptibility testing is used to select effective antimicrobial drugs susceptiblity testing is not usually indicated when the sensitivity reactions of a pathogen can be predicted, for example:

-*Proteus* species are generally resistant to nitrofurantoin and tetracyclines

-*S.pyogenes* is usually sensitive to penicillin, *K.pneumoniae* is generally ampicillin resistant.

- Anaerobes are sensitive to metronidazole, sensitivity tests must never be performed on commensal organisms or contaminants because this

would mislead the clinician and could result in the patient receiving ineffective and unnecesary antimicrobial thearpy, causing possible side effects and resistance to other potentially pathogenic organisms (Cheesbrough, 2004). Such information forms the basis for best guess thearpy when patients have to be treated before laboratory results are available. The ssusceptibility pattern may also help in identification of infecting organism **(**Hawkey and Lewis, 1989)

1.2.4.1 Susceptibility testing techniques: -

Antimicrobial susceptibility testing can be performed using: -

-A dilution technique.

- A disc diffusion technique.

1.2.4.1.1 **Dilution sensitivity tests**: Manual or semi-automated dilution sensitivity tests are performed in microbiological reference laboratory for epidemiological purposes or when a patient does not respnod to treatment thought to adequate, relapses while being treated or when there is immunosupression. Dilution techniques measure the minimum inhibitory cocentration (MIC). They can also be used to measure the minimum bacterial cocentration (MBC), which is the lowest concentration of antimicrobial required to kill bacteria.

Adding dilutions of an antimicrobial to a broth or agar medium carries out a dilution test. A standardized inoculum of the test organism is then added. After over night incubation, the MIC is reported as the lowest concentration of antimicrobial required to prevent visible growth. By comparing the MIC value with known concentrations of the drug obtained in serum or other body fluids, the likely clinical response can be assessed. When required the MBC can be determined by subculturing the

last tube to show visible growth and all the tubes in which there is no growth (Cheesbrough, 2004**).**

1.2.4.1.2 Disc diffusion susceptibility tests: -

The disc diffusion method is the technique most commonly used for routine antimicrobial susceptibility testing .The method is convenient, technically simple, cheap, and, if correctly performed, reasonably reliable. The surface of an agar plate is evenly inoculted with organism and a filter paper disc containg a defined amount of antimicrobial agent is applied to the inoculated plate .After incubation (usually over night at (35-37 °C) there is a circular zone of inhibition around the disc as a result of diffusion of the agent into the agar and inhibition of growth of the organism .The size of the zone of inhibition is an indication of the susceptibility of the organism. More resistant organisms giving small zone sizes, the size of the zone of inhibition is, however, influensed by technical variables that must be controlled to produce meaningful results. The theoretical aspects of zone formation developed by Cooper and Linton have been interpreted in relation to more recent diffusion procedures described by Barry (1986).

1.2.4.2Factors affecting diffusion test: -

These have been extensively reviewed and summarized below:

1.2.4.2.1 **Choice of medium:** The culture medium should support the growth of organism normally tested without being antagonistic to the activity or diffusion of agents. Some of the factors influencing the activity of various antibiotics are shown in Table (4).

1.2.4.2.2 Depth of medium: Zones of inhibition increase as the depth of agar decreases, and the effect is more marked with very thin plates. Plates should therefore have a constant level depth of 3-4 mm.

1.2.4.2.3 Inoculum density: Increasing the inoculum size reduces zone size with all antimicrobial agents to some extent. Variation in inoculum size is one of the main sources of error in susceptibilty testing. Most disc diffusion methods recommended an inoculum resulting in semi- confluent growth of colonies. This has the advantage that an incorrect inoculum can be seen and the test repeated .The inoculum is generally acceptable if the density is between almost confluent and colonies separated to the extent that zones cannot be measured **(**Hawkey and Lewis 1989).

1.2.4.2.4 Pre- incubation and pre-diffusion: -

Pre-incubation of inoculated plates before discs are applied reduces zone size. And pre-diffusion of antimicrobial agents prior to incubation has the opposite effect. Athough a set prediffusion time of 30-60 min may improve reproducibility of tests; attempts to standardize pre-incubation and pre-diffusion times present practical difficulties.

1.2.4.2.5 Antimicrobial discs: -Commercially produced filter paper discs are almost universally used. Although problems with the discs are occasionally due to manufacturning failures, most faults are related to inadequate handling of discs in the laboratory. . High temperture, and particularly high humidity, lead to more rapid deterioration of labile agents, especially β-lactams. Discs should therefore be stored and handled in optimumm conditions.

1.2.4.2.6-Incubation: Plates are incubated at 35-36°C in air unless another atomosphere is essential for growth. An atmosphere containing

additional carbon dioxide should be avoided because this reduces the pH and thus may give false result with some agents. Stacks of plates should be as small as possible, preferably no more than5 plates high, as plates in the center of large stacks take considerably longer to warm to incubator temperature than at top and bottom.

1.2.4.2.7 Reading of zones: - Reproducibility of reading zones is related to the clarity of zone edges. Hence the reading of tests on sulphanomides and streptococci tends to be most variable. Generally there is an obvious zone edge. Small colonies or a film of growth at zone edges, swarming of *Proteus*, spp, into zones, or haemolytic effects on media should be ignored. If it is necessary to measure zones, calipers (preferably) or a ruler should be used (Hawkey and Lewis, 1989). For clinical and surveillnace purposes and to promote reproducibility and comparability of results between laboratories, WHO recommends the National Committee for Clinical Laboratory standard (NCCLs) modified Kirby-Baur disc diffution technique. The validity of this carefully standardized tehcnique depends on using disc of correct antimicrobial content, an inoculum that gives confluent growth, and a reliable Mueller-Hinton agar. The test method must be followed exactly in every detail. After incubation at 35°C for 16-18 hours, zone sizes are measured and interpreted using NCCLs standards. These are derived from the correlation, which exists between zone size and MICs (Cheesbrough**,** 2004).

Table -1 Mechanism of Bacterial Resistance to Antimicrobial Agents

Type of Defect	**Example**	**Mechanism**
Inadequate intery into bacterial cell	Aminoglycoside and *Pseudomonas species*	Poorly understood defects in "permeases" or other transport mechanism, especialy in Gram-negative bacteria
Enzymatic inactivation of antibiotics	B-lactamses and Various bacteria Aminoglycosides and Gram-negative bacteria Chloramphenicoal and *S.aureus* or Gram-negative bacteria	Chromosomal-and or plasmid-coded enzymes varying specificties for penicillins and cephalosporins (eg, *Staphylococcus aureus, Haemophilus influenzae, and Neisseria gonorrhoeae)* Several enzymes affecting acetylation, phosph orylation, and adenylyation. Aminoglycosides vary in susceptibility to the enzymes. Plasmid-coded acetyltransferases are responsible for most resistance to this drug. The enzyme is constitutive in gram-negatives but induced by the antibiotic in *S.aureus*.
Alteration of penicillin-binding proteins (PBP)	B-lactam antibiotics and Gram-positive bcteria	Intact PBPs are necessary for the activity of B-lactam drugs.Changes in these proteins cause the multiple resistance in sterptococcus pneumoniae and probly methicillin resistance in S.aureus.
Alteration of ribosomes	Erythromycin and *S. aureus*	Plasmid- mediated methylation of the 30s ribosome blocks attachment of the drug to the ribosome.

Warren & Ernest, 2002

Table 2 Mechanisms of action of important antibacterial & antifungal drugs

Mechanism of Action	Drugs
Inhibition of cell wall synthesis **1- Antibacterial activity** Inhibition of cross-linking (transpeptidation) of peptidoglycan Inhibition of othe steps in peptidoglycan synthesis **2-Antifungal activity** Inhibition of B hlucan synthesis	 Penicillins, cephalosporins, imipenem, aztreonam, vancomycin. Cycloserine, bacitracin. Caspofungin
Inhibition of protein synthesis Action on 50s ribosomal subunit Action on 30s ribosomalsubunit	 Chloramphenicol,erythromythin, clindamycin, linezolid Tetracyclines and aminoglycosides.
Inhibition of nucleic acid synthesis Inhibition of nucleotide synthesis Inhibitin of DNA synthesis Inhibition of mRNA synthesis	 Sulfonamides, trimethoprim Quinolones Rifampin
Alteration of cell membrane function Anti bacterial activity Antifungal activity	 Polymyxin AmphotericinB,nystatin,ketoconazole
Other mechanism of action 1- Antibacterial activity 2- Anti fungal activity	 Isoniazid,metronidazole, ethambutol, pyrazinamide Griseofulvin,pentamidine

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Table 3 Mode of action of protein synthesis. Inhibitor antibiotics

Antibiotic	**Ribosomal subunit**	**Mode of action**	**Bactericidal or bacteriostatic**
Aminoglycosides	30s	Blocks functioning of initiation complex and causes misreading of mRNA	Bactericidal
Tetracyclines	30s	Blocks tRNA binding to ribosome	Bacteriostatic
Chloramphenicol	50s	Blocks peptidyltransferase	Both
Erythromycin	50s	Blocks translocation	Primarily bacteriostatic
Clindamycin	50s	Blocks peptidebond formation	Primarily bacteriostatic
Linezolid	50s	Blocks early step in ribosome formation	Both

(Warren and Ernest 2002**)**

Table 4. Factors affecting antimicrobial activity on culture media

Factor	Agent affected	Effect on activity
Thymidine	Sulphonamides Trimethoprin	Reduced
Rasied pH	Aminoglycosides Macrolides Lincosamides Nitrofurantoin	Increased
Lower pH	Tetracycline Methicillin Fusidic acid Novobiocin	Increased
Monovalent cations (eg. Na+)	Bacitracin Fusidic acid Novobiocin Penicillin Tetracycline	Increased against staphylococci Increased against *Proteus* spp Reduced
Divalent cations (eg. Mg2+ and Na2+)	Polymyxins Aminoglycosides	Reduced against *Pseudomonas* spp.

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1.2.5 Wound infection: -

Wound infections follow surgery or trauma that disrupts the skin or mucosal surface (e. g. road accidents and bites). Post –operative wound infections commonly follows gastro-intestinal surgery and some gynaecological surgery (Bushell, 1989**).** It is so difficult to list all pathogens that may be found in pus (Cheesbrough, 2004) .The type of infecting organsim depends on the site and nature of the surgery or trauma **(**Bushell, 1989**)**. Woud infections following colo- rectal surgery often contain bacteria from the large bowel ***(****E.coli*, *Bacteroides* spp. etc.). Wound infections follow bites will contain mouth organisms from the biting animal. Wound infections are often caused by organisms resident on the skin surface which has been breashed. The main culprit here is *Staphylococcus aureus*, and this is the only constituent of the normal skin flora that is worth looking for routinely.

i) Common: *S. aureus*, β-haemolytic *Streptococcus*, *E.coli*, *Bacteroides fragilis*, *Proteus* spp, other *Enterobacteriaceae*, *CL*-perfringens, anaerobic cocci.

ii) Less common: mirco-aerophilic *Streptococcus*, *Pasteurella multocida* (animal bites) other *Clostridia,* other *Bacteroides, Fusobacterium* spp, *Pseudomonas spp*, *Salmonella* spp, *Capnocytophaga canimrsus* (formerly *D FZ).*

iii) Rare: *Vibrios* (infected marine wounds).*Cl.tetani,*fungi (Bushell,1989)

Note on pathogens: *S.aureus* is the commonest pathogen isolated from subcutaneous abscesss and skin wound. It also causes imetigo (small pustules that form yellow crusty sores, usually around the mouth).

Penicilln and methecillin resistant strains of *S.aureus* are common causes of hospital-acquired wound infections.

Ps.aeruginosa is associated with infected burns and hospital acquired infections.

E.coli, *Proteus* spp, *Pseudomonas aeruginosa*, and Bacteroides spp are the pathogens most frequently isolated from abdominal abscesses and wounds .Pus containing *Bacteroide**s*** spp has a very unpleasant smell (as also pus containing other anaerobes).

*Cl.perfringens*is found mainly in deep wounds where anaerobic conditions exist. The toxins produced cause putrefactive decay of the infected tissue with gas production .The death and decay of tissue by *Cl.perfringens* is called gas gangrene.

Chronic leg ulceration is common in those with sickle cell disease. The commonest pathogens isolated are *S, aureus. Ps.aeruginosa, S. pyogenes*, and Bacteroides species.

Mycobacterium tuberculosis is associated with cold abscesses.

Actinomycetes filamentous bacteria and several species of fungi cause mycetoma. Specimens of pus from the draining sinuses contain granules. Examination of which helps to differentiate wheather the *Mycetoma* is bacterial (treatable) or fungal (less treated).

Actinomycetes israeli and other species of actinomyces cause actinomycosis. Small yellow granules can be found in pus from draining sinuses (often in the neck).

Vincent's organsims (*Borrelia vincenti* with Gram- negative anaerobic *Fusiform bacilli**)*** are asociated with tropical ulcer. The ulcer is commonly

found on the leg. Often of malnourished persons, especially children**.** *Staphylococcus* and S*treptococcus* are frequently secondary invadors (Cheesbrough, 2004).

1.2.6 Normal wound healing: -

The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. This overview will help in identifying the various phases of wound healing (Mathieu *et al.,* 1999). Healing of wounds, whether from accidental injury or surgical intervention involves the activity of intricate network of blood cells, tissue types, cytokines, and growth factors .This results in increased cellular activity, which causes an intensified metabolic demand for nutrients (Thom *et al*., 1997) .Nutritional deficiencies can impede wound healing, and several nutritional factors required for wound repair may improve healing time and wound outcome.Vitamin A is required for epithelial and bone formation, cellular differentiation, and immune function .Vitamin C is necessary for collagen formation, proper immune function, and as a tissue antioxidant. Vitamin E is the major lipid- soluble antioxidant in the skin; however, the effect of vitamin E on surgical wounds is inconclusive. Bromelain reduces edema, bruising, pain, and healing time following trauma and surgical procedures. Glucosamine appears to be the rate- limiting substrate for hyaluronic acid production in the wound. Adequate dietary protein is absolutely essential for proper wound healing, and tissue levels of the aminoacids arginine and glutamine may influence wound repair and immune function (Douglas *et al*., 2003)**.**

The botanical medicines *Centella asitica* and *Aloe vera* have been used for decades, both topically and internally, to enhance wound repair, and

scientific studies are now beginning to validate efficacy and explore mechanisms of action for these botanicals. To promote wound healing in the shortest time possible, with minmal pain, discomfort, and scarring to the patient. It is important to explore nutritional and botanical influences on wound out come (Douglas *et al.,* 2003).

Wound repair or restoration of tissue integrity is natural reaction to injury. Many drugs of natural or chemical origin (e.g. quince seed mucilage and Ginko bilba, phenytoin, Zink oxide, Ketanserin, dexpanthenol) have been used topically (Arzi *et al.,* 2003).

1.2.6.1 Phases of wound healing: -

1-Inflammatory phase

A) Immediate to 2-5days.

B) Hemostasis: -

Vasoconstriction.

Platelet aggregation.

Tromboplastin makes clot.

C) Inflammation:

Vasodilation.

Phagocytosis.

Proliferative phase

A) 2 days to 3 weeks.

B) Granulation

Fibroblasts lay bed of collagen.

Fills defect and produce new capillaries.

C) Construction

Wound edges pull together to reduce defect

D) Epithelization

Crosses moist surface

Cell travel about 3 cm from point of origin in all directions.

Remodeling phase

A.) 3weeks to 2 years

B) New collagen forms which increases tensile strength to wounds.

C) Scar tissue is only 80 percent as strong as original tissue (Thom *et al*., 1997).

Tissue injury initiates a response that first clears the wound of devitalized tissue and foreign material, setting the stage for subsequent tissue healing and regeneration. The intial vascular response involves a brief and trasient period of intese vasoconstriction and hemosttasis 5-10 minute; period of intense vasoconstriction is followed by active vasodilation accompanied by an increase in capillary permeability. Platelets aggregated whithin afibrin clot secrete a variety of growth factors and cytokines that set the stage for an orderly series of events leading to tissue repair (Douglas *et al*., 2003).

The second phase of wound healing the inflammatory phase, presents itself as erythema, swelling, and warms, and is often associated with pain

.The inflamatory response increase vascular permeability,resultingin migration of neutrophils and monocytes into the surrouding tissue .The neutrophils engulf debris and microorgnisms, providing the first line of defence against infection .Neutrophil migration ceases after the first few days post- injury if the wound is not contaminated If this acute inflammatory phase persist, due to wound hypoxia, infection ,nutritional deficiencies, medication use, or other factors related to the patient immune response, it can interfere with the late inflammatory phase (Douglas *et al*., 2003).

1.2.7 Biochemical tests for identification of bacteria:-

While several commercial systems for identifying bacteria are available, these are often difficult to obtain or too expensive to use in developing countries (Cheesbrough, 2004). However, the following of conventional biochemical tests are used for identification of bacteria.

1.2.7.1 Gram reaction: -

Gram did not describe a stain but a method in which he used stains and solutions devised by others, to this day we do not fully understand its mechanism, but we do know that the reaction to Gram stain method is stable charateristic of bacterium. Gram positivity (the ability to resist decolorization with ethanol or acetone) is a feature of relatively young bacterial cells of some species, as they age, the cells loose this characteristic and apparetly become Gram –negative. It is important, therefore, to examine young cultures, preferably before the end of the logarithmic growth phase. Arecent modification by Preston and Morrell is claimed to be fool proof (Cowan and Stell 1970).

Morphology: May be affected by the medium on which the organism is grown and the temperture of incubation (Cowan and Stell 1970)

1.2.7.2 Catalase test: -

This is used to differentiate bacteria that produce the enzyme catalase, such as *Staphylococcus*, from non –catalase producing bacteria such as *Streptococcus*.Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxgen and water .An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxgen are relased if the organism is a catalase produser .The culture shoud not be more than 24 hours old (Cheesbrough, 2004**)**

1.2.7.3 Citrate utilization test: -

This test is one of several technique used occasionally to assist in the identification of Enterobacteria .The test is based on the abilityof an organism to use citrate as its only sourse of carbon. There are two ways for performing this test. Using a rasco citrate identification tablet. This is the most economical method when only a few tests are performed. The tablets have along shelf life and good stability in tropical climates.

Using Simmon citrate agar but the dehydrated medium is only availble in 500g-pack size from manufactures. After being opened the medium does have good stability in tropical climates.

1.2.7.4 -Coagulase test: -

This test is used to identify *S.aureus* which produces the enzyme coagulase causes plasma to clot by converting fibrinogen to fibrin.

Two types of coagulase are produced by most strain of *S.aureus*.

Free coagulase which convert fibrinogen to fibrin by activating a coagulase- reacting fator present in plasma. Free coagulase is detected by clotting in the tube test.

Bound coagulase (clumbing factor) which converts fibrinogen directly to fibrin without requiring a coagulase –reacting factor .IT can be detected by the clumping of bacterial cells in the rapid slide test. A test tube most always be performed when the result of a slide test is not clear, or when the slide test is negative and *Staphylococcus* has been isolated from a serious infection. A tube test is reguired to detect some MRSA (methicillin resist *Staphylococcus aureus* strains. Before performing a coagulase test, examine a Gram stained smear to confirm that the organism is a Gram-positive cocci (Cheesbrough, 2004).

1.2.7.5 DNase test: -

This test is used to identify *Staphylococcus aureus* which produses dexoyribonucease enzyme. The Dnase test is particularly used when plasma is not available to perform coagulase test or when the result of the coagulase test is difficult to interpret

Deoxyribonuclease hydrolysis deoxyribonucleic acid (DNA). The test organism is cultured on a medium, which contains DNA. After over night incubation, the colonies are tested for Dnase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates hydrolyzed DNA. Dnase –producing colonies are therefore surrounded by clear areas due toDNA hydrolysis (Hawkey and Lewis 1989).

1.2.7.6 Indole test: -

Testing for indole production is important in the identification of Enterobacteria. Most strains of *E.coli*, *P.vulgaris*. *P.rettgeri, M.morganii*

and *providencia* species break down the aminoacid tryptophan with the release of indole .The test organism is cultured in a medium, which contains tryptophan. Indole production is detected by Kovac or Elrlich's reagent, which contains 4(p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red coloured compound. Kovac's reagent is recommended in preference to Ehrlichs reagent for the detection of indole from Enterobacteria (Cheesbrough, 2004).

1.2.7.7 Oxidase test: -

The oxidase test is used to assist in the identification of *Pseudomonas*, *Neisseria*, *Vibrio, Brucella*, and *Pasteurella* species, all of which produce the enzyme cytochrome oxidase .A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used .If the organism is oxidase –producing, the phenyleneiamine in the reagent will be oxidized to deep puple colour. Occasionally the test is performed by flooding the cultue plate with oxidase reagent but this technique is not recommended for routine use because the reagent rapidly kills bacteria. It can however be useful when attempting to isolate *N.gonorrhoeae* coloies from mixed culture in the absence of a selective medium .The oxidase positve colony must be removed and subcultured within 30 seconds of flooding the plate. Acidity inhibits oxidase enzyme activity .The oxidase test must not be performed on colonies that produce fermentation on carbohydrte-cotaining media such as TCBS or MacConkey agar. Subculture on nutrient agar is required before the oxidase test can be performed. Colonies tested from a medium that contain nitrate may give unreleable oxidase test results *(*Hawkey and Lewis 1989).

1.2.7.8 Urease test: -Testing for urease enzyme activity is important in differentiating Enterobacteria. *Proteus* strains are strong urease producers**.***Y.enterocolitica* also shows urease activity (weakly at 35-37°C). *Salmonellae* and *Shigella**e*** do not produce urease. The test organism is cultured in a medium contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will brake down urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red (Cheesbrough, 2004).

1.2.7.9-Carbohydrate utilization test: -The so-called"fermentation tests"were used by the early bacterilogists to distinguish one organism from another and elaborate diagnostic tables were based on them. The introduction of the simple gas tube (Durham, 1898) and indicators enabled the production of gas and acid to be detected by inspection (Cowan, 1970). Peptone water, sugars and Rasco sugar fermentation tablets are used to identify bacteria by their fermentation reactions (Cheesbrough, 2004).

1.2.7.10 The MR (Methyl red) test and V-P (Voges–Proskauer) test for acetylemethylcarbinol or acetoin may be carried out on the same tube of culture and are discussed together. The tests mainly used to distinguish various *Coliform* organisms from each othor; all ferment glucose vigrously and the pH value of the glucose medium falls quickly. When methyl red is added after overnight incubation the cultures of all these organisms will be found to be acid to the dye, i.e.MR positive. After further incubation *Escherichia coli* cultures produce even more acid and inspite of phosphate buffer in the medium may be self-sterilizing; The MR test remains positive. *Klebsiella pneumoniae* cultures, on the other

hand, decarboxylate and condense the pyruvic acid to form acetylmethylcarbinl, the pH value rises and, when methyl red is added, the colour is yellow. i.e. MR –negative. Nowadays there is a tendency to do biochemical tests earlier but the temptation to speed up the MR test must be resisted, the MR shoud never read until the cultures have been incubated for at least two days at 37°C or three days at 30°c. The VP test can be obtained. It is now generally thought that the older methods are too slow and insenstive, but there is less agreement about the method to be recommended or the senstivity that gives the best differentiation between species (Cowan and Stell 1970**)**

1.2.7.11 Pigment production: -

Often has considerable diagnostic value and it is an advantage to know how to induce it. Although the pigments produced are seldom photosynthetic, most bacteria dealt within laboratories form pigment better in the light; this is most noticeable in *Staphylococcus* and *Serratia***,** and also occurs in the *Pseudomonas* and in Chromobacteria. The effect of light on pigment production by bacteria has become a means of distinguishing species. Temperatures and medium also influence the intensity of pigmentation; most bacteria produce pigments better at temperatures below the optimum for growth. Medium probably has the biggest effect on the development of pigment. In some cases the addition of glucose will enhance pigmentation; in other cases this will inhibit it. (Hawkey and Lewis 1989).

1.2.7.12 -Kligler iron agar: -KIA reactions are based on the fermentation of lactose and glucose (dextrose) and the production of hydrogen sulphide. A yellow but (acid production) and red- pink slope indicate the fermentation of glucose only. The slope is pink-red due to a

revertion of the acid reaction under aerobic conditions. This reaction is seen with *Salmonella* and *Shigella* species and other enteric pathogens cracks and bubles in the medium indicate gas prodution by *S. paratyphi* and some faecal commensals. A yellow slope and a yellow butt indicate the fermentation of lactose and possiblly glucose. This occurs with *E.coli* and other Enterobacteria**.** Ared –pink slope and butt indicate no fermention of glucose or lactose. This is seen with most strains of *Ps.aeruginosa*. Blackening along the stab line or throughout the medium indicates hydrogen sulphide (H_2S) production e.g. *S.typhi* produces a small amount of blackening whereas *S.typhimurium* causes extensive blackening (Cheesbrough, 2004).

1.2.7.13 Novobiocin disc:

This senstivity test is used to differetiate between *Staphylococcus* species (Cheesbrough, 2004).

1.2.7.14 Growth at 42°C: -

Differentiates *Ps.aeruginosa* from the less commonly isolated *Pseudomonads*, *P.putida* and *P.fluorescens* (Cheesbrough, 2004).

1.2.8 Selction of the appropriate laboratory animals:

Animals are used to show standard response to experimental manipulation. Animals must be of the same: species, subspecies, strain, and sex, the same age range, reared under similar conditions and not subject to previous experimental interference. One must try to achieve maximum accuracy with the minimum number of animals. Rodent animals can be used in the preliminary screening experiments.

Swiss Wistar Albino rats are popular for their size, ease of handling, low cost of housing, short life span, the broad similarity to human physiology and usefulness in wound healing evaluation experiments (Poole and Robinson 1989).

1.2.9 Ointments: -

Ointments are semisolid preparations intended for external application to the skin or mucous membrane. They may be medicated or non-medicated.

Non-medicated ointments are used for the physical effects that they provide as protectants, emollients or lubricants. Ointment bases, as described may be used for their physical effects or as a vehicle in the preparation of medicated ointments (Ansel *et al*., 2002).

1.2.9.1 Ointment bases:-

Ointment bases are classified by the United State Pharmacopea (USP) into four general groups:

i) Hydrocarbon bases.

ii) Absorption bases.

iii) Water removable bases.

iv) Water soluble bases.

1.2.9.1.1 Hydrocarbon bases:

Also termed oleaginouss bases on application to the skin:

i) They have an emollient effect.

ii) Protection against the scape of moisure.

iii) Are effective as occlusive dressings.

iv) Can remain on the skin for prolonged periods of time without drying out.

v) Due to immiscibility with water are difficult to wash-off.

vi) Water and aqueous preperations may be incorporated into them, but only in small amounts and with some difficulty.

Petrolatum, white petrolatum, white ointment and yellow ointment are examples of Hydrocarbon bases.

1.2.9.1.1.1. Petrolatum USP:

Petrolatum, USP is a purified mixture of semisolid hydrocarbons obtained from petroleum. It is an unctuous mass, varying in colour from yellowish to light amber.

It melts at temperature between 38°C and 60°C and may be used alone or in combination with other agents as an ointment base. Petrolatum is also known as "yellow petrolatum and petroleum jelly". A commercial product is Vaseline'Chesebrough-Ponds' **(**Ansel *et al*., 2000).

1.2.9.1.1.2 Liquid paraffin:

This is a mixture of liquid hydrocarbons obtained from petroleum. It is a transparent, colourless, almost odorless oily liquid. On long storage it is liable to oxidation with production of peroxides and therefore it may require an antioxidant, e.g. Tocopherol or butylated hydroxytoluene (BHT). It is used to soften ointment bases and to reduce the viscosity of creams (Collet and Aulton, 1991).

1.2.9 1.2 Absorption bases

They are two types:

i) Those that permit the incorporation of aqueous solutions resulting in the formation of water in oil emulsions (e.g. hydrophilic petrolatum).

ii) Those that are water in oil emulsions (syn.: emulsion bases) and permit the incorporation of additional quantities of aqueous solutions (e.g. Lanolin).

These bases may be used as emollients although they do not provide the degree of occlusion afforded by the hydrocarbon bases. Absorption bases are not easily removed from the skin with water washing since the external phase of the emulsion is oleaginous (Ansel *et al.,* 2002).

1.2.9.1.2.1 Lanolin, USP:

It is obtained from the wool of sheep (Ovis Aries) is a purified, wax-like substance that has been cleaned, deodorized and decolorized. It contains no more than 0.25% water. Additional water may be incorporated into lanolin by mixing.

1.2.9.1.3 Water removable bases (water miscible):

i) Oil-in-water emulsions resembling creams in appearance.

ii) Because the external phase of the emulsion is aqueous, they are easily washable from skin and are often called “water washable bases”

iii) They may be diluted with water or aqueous solutions.

iv) They have the ability to absorb serous discharges.

Hydrophilic ointment, USP is an example of this type of bases (Ansel *et al.,* 2002)

The three emulsifying ointments from water-miscible bases, i.e.

Emulsifyingg ointment BP (anionic), cetrimide emulsifying ointment BP (cationic) and cetomacrogol emulsifying ointment BP (non-ionic)

These contain paraffins and an o/w emulgent and have the general formula:

Anionic, cationic or non-ionic emulsifying Wax	30 %
White soft paraffin	50 %
Liquid paraffin	20%

They are used for preparing o/w creams (Collett and Aulton, 1991).

1.2.9.1.4 Water-soluble bases:

They do not contain oleaginous components. They are completely water washable and often referred to, as 'Greaseless' because they soften greatly with addition of water, large amounts of aqueous solutions are not effectively incorporated into these bases. They are mostly used for the incorporation of solid substances. Polyethlene glycol ointment, natural formula (NF) is the prototype example of water-soluble base.

1.2.9.1.4.1 Polyethlene glycol ointment (natural formula) (macrogol or carbowaxes):

Polyethlene Glycol (PEG) is a polymer of ethylene oxide and water presented by the formulation H (OCH2CH2) n OH in which n represents the average number of oxyethlene groups. The numerical designations associated with PEGs refer to the average molecular weights of the polymer. PEGs having average molecular weights below 600 areclear, colorless liquids, those with molecular weights above 1000 are wax like white materials, and those with molecular weights in between are semisolids. The greater the molecular weight the greater is the viscosity

.The natural formula (NF) lists the viscosities of PEGs ranging from average molecular weights of 200 to 8000. The general formula for the preparation of 1000g of polyethene glycol ointment is:

Polyethlene glycol 3350	400grams
Polyethlene glycol 400	600grams

Shayoub (1985) listed the advantages and disadvantages of Polyethylene Glycol.

1.2.9.1.4.1.1 **Advantages of Polyethlene Glycol**:

1/ PEGs vehicles form non-occlusive films on skin.

2/ PEGs are anhydrous and can easily wash from the skin.

3/ Good absorption by the skin.

4/ Good solvent properties. Some water- immissible dermatological drugs such as hydrocortisone, salicylic acid, sulphonamides, sulphur and redsonoic acid are soluble in macrogols.

5/ Freedom from greasiness.

6/ Satifactory agening properties. They do not hydrolyzse, rancidify or support microbial growth.

7/ Compatibility with many deratological medicaments e.g. ammoniated mercury; yellow mercuric oxide, icthammol and sulphur (Robinson *et al*., 1964).

1.2.9.1.4.1.2 Disadvantages of Polyethylene Glycol:-

1/ Limited uptake of water, macrogols dissolve when the proportion of water reaches about5% bases may be thinned with liquid macrogol or

with propylene glycol. Inclusion of a higher fatty alcohol, such ascetostearly, which allows incorporation of larger quantites of water, and improves the texture of the bases (Nexon, 1951 cited in Shayoub 1985).

2/ They are less bland than paraffins, possibly due to their hygroscopic nature.

3/ They cause reduction in activity of certain antibacterial agents,e,g. phenols. Hydroxyl benzoates and quaterary ammonium componunds **(**Pattel and Foss, 1964). Some antibiotics are rapidly inactivated such as penicillin and bacitracin (Couster *et al*., 1961).

4/ They have a solvent action on polyethlene and backelite; these plastics should not be used as containers or closures for macrogel ointments.

1.2.9.2 Properties of the Ideal Base:

1- It does not retard wound healing.

2-It has a low sensitization index.

3/ It must be neutral.

4/ It has a non-dehydrating effect.

5/ It has an acceptable pharmaceutical elegance.

6/It must be compatible with common medications.

7/ It has a good keeping quality.

8/ It has alow index of irritation.

9/ It has non-greasy property.

10/ It has a minimum number of ingredients.

11/ It has an efficient release of medicament at the site of application.

12/ It is washable.

13/ It has an easy compounding property (Shayoub, 1985).

1.2.9.3 Selection of the appropriate base:

The selection of the base to be used in formulation of an ointment depends upon the careful assessment of a number of factors inculding:

1/ The desired release rate of the drug substance from the ointment base.

2/Desirability for topical or percutaneous drug absorption.

3/Desirability of occlusion of moisture from the skin.

4/ Stability of the drug in the ointment base.

5/ Effect.if any, of the drug on the consistency or other features of the ointment base.

6/ The desire for base that it is easily removed by washing with water.

The base that provides the majority of the most desired attributes should be selected (Ansel *et al*., 2000).

1.2.9.4 Compounding of Ointments and pastes:

The basic techniques for the preparation of ointments and pastes are:

Weighing, measuring of liquids, size reduction and size separation, and mixing.

1.2.9.4.1 Fusion mixing method: -

In this method, the ingeredients are melted together and stirred to ensure homgeneity, on a small scale; fusion is usually carried out in an evaporating basin over a water- bath. Stainless basins are to be preferred.

1.2.9.4.1.1Preparation of the Ointment base by fusion:

The constituents of the base should be placed together in the basin and allowed to melt together. Melting time is shortened if high melting point ingerdients, such as hard paraffin and the emulsifying waxes are grated into the basin and heated while other ingerdients are being prepared.

After melting, the ingerdients should be stirred until cool.

1.2.9.4.1.2 Preparation of Medicated Ointments and Pastes by Fusion:

Solid that is completely or partialy soluble in the base should be added in fine powder to the molten base at a low temperature as possible and the mixture stirred until cooled **(**Collett and Aulton, 1991).

1.2.9.5 Application frequency:

Topical agents are often applied twice daily (Hardman *et al.,* 1996).

1.2.9.6 Microbial contents:

With the exception of ophthalmic preparations, topical applications are not required to be sterile. They must, however, meet acceptable standards for microial contents. Preparations which are prone to microial growth must be presented with antimicrobial preservatives (Ansel *et al*., 2000).

1.2.10 The plants used in this study are: -

1.2 10 .1 *Guiera senegalensis:* -

Vernacular name: Gubeish (Arabic).

Family: Combretaceae **(**Elghazali, 1997).

Botanical description: Grey tomentose shrubs up to 3m high. Leaves opposite, or subopposite elliptic- oblong, 6-12 x 0.5 - 2.8 cm, apex mucronate, base slightly cordate to attenuate, margin entire. Inflorescences dense terminal heads up to 2 m across. Fruit capsules, spindle- shaped, angled, 2.5-3.0 cm, grey-brown (Elghazali, *et al*., 1987).

Habitat: Sandy lowland plains, degarded savanna.

Distribution: Tendelti, Rashad, Wad ashana and central Sudan.

Chemical constituents: Flavonoids, saponins, alkaloids, mucilages and tanins100 were isolated (Elghazali, 1997). A new methoxylated naphthyl butenone, guieranone A were isolated from the leaves of *Guiera senegalensi* and it is the first derivative that has been extracted from the family Combretaceae.

Uses: The macerations of the leaves used as antidiabetic, antipyretic, anti-vomiting and antileprosy (Elghazali, 1997). In African traditional medicine the leaves are also used for gastrointestinal disorders, coughs and topically for wound healing (Bosiso *et al*., 1997). Plant is used for malaria in Mali and Sao-tome traditional medicine (Ancolio *et al*., 2002).

1.2.10.2 *Kigelia africana* (Lam.) Benth.

Common name: Sausage tree**.**

Vernacular names: - Abu shutour, Umm Mashatour (Arabic).

Family: Bignoniacsae.

Botanical description: Large savanna trees up to 15 m high. Leaves imparipinnate up to 30 cm long; Leaflets 7-9, opposite, sessile to subsessile, obtuse, 4-7 x1.8-3cm, apex rounded to mucronate, base cuneate, margin undulate. Inforescences laxpanicles up to 50 colonyg. Fruit berries, sausage –shaped, up to 50 cm long, pale green.

Habitat: Khor, river banks and vallies.

Distribution: Wide spread.

Chemical constituents: A bitter principle and tannic acids were isolated from the bark. Different morphological parts of *Kigelia* were shown to contain dihydro-isocoumarins, 6 methoxy mellein *p*innata, terpenoid aldehyde, an iridoid glycoside (veratraldehyde) and naphthaquinone (Elghazali *et al*., 1987).

Uses: Traditionally used in West Africa for wounds and abscesses. The aqueous extract of the bark is used for backache, stomach pains and dysentery. Plant is used as antimalaria, febrile jaundice, menorrhagia. (Elghazali *et al.,*

2. MATERIALS AND METHODS

2.2 Methods

2.2.1. Identification of the clinical isolates:

One hundred clinical isolates of *Escherichia coli***,** *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus,* were collected randomly during the period from June - December 2005 from National Health Laboratory, Khartoum and Omdourman Educational Hospitals.These clinical isolates were obtained from, urine, wounds, ear swab, abscesses and eye swab. Streaking on mannitol salt agar, MacConkey agar crystal violet blood agar, cetrimide agar and cooked meat medium purified them. They were identified on the basis of the results of microscopical examination (Gram stain), cultural characteristics and biochemical tests (Cruickshank *et al.*, 1975).

Media used for identification of clinical isolates:

All specimens were inoculated on two blood agar plates, one incubated aerobically and the other anaerobically using gas-generating kits. The obtained isolates were then purified by streaking on mannitol salt agar plates, MacConkey's agar crystal violet agar, cetrimide agar and cooked meat medium.

The purified isolates were then subcultured on mannitol, MacConkey agar, crystal violet blood agar, cetrimide agar and cooked meat medium slopes and then stored in a refrigerator until they were used.

2.2.1.1 Microscopical examination of aerobic bacterial isolates.

All isolates were subjected to microscopical examination to study their staining properties (using the Gram's staining technique), the shapes and cellular arrangements.

The bacterial film is fixed then flooded with crystal violet for one minute, then the stain washed off with clean water and coverd with iodine for one minute and decolorized rapidly with acetone- alcohol, washed clearly with clean water, decolorized smears coverd with safranin for two minutes and then examined microscopically.

2.2.1.2 Simplified routine biochemical tests for identification of bacterial isolates:-

The biochemical activities of the purified isolates were then studied for identification and confirmation of these organisms. The biochemical tests carried out include:-

2.2.1.3.1 Fermentation tests:

Different bacteria are variously able to ferment carbohydrates (e.g. glucose, lactose, and sucrose). Sterile peptone water was used, with 1 % of the sugar, and suitable pH indicator, is inoculated with the test organism and incubated at 37ºC for 1-3 days. Acid production is detected by a colour change of the indicator, and gas production by an inverted Durham′s tube, which has been completely filled with the medium (with phenol red the colour changed from pink to yellow indicating fermentation and production of acid). The production of gas was detected by the presence of air-bubbles in the tube (Harris, 1964).

2.2.1.3.2. Methyl red tests:

It is used to detect the ability of some bacteria to produce sufficient amounts of acidic substances due to fermentation of glucose using methyl red- Voges- Proskauer medium. Colour changes from yellow (pH 6.2) to red (pH4.2) with acid production on addition of methyl red indicator. (Cruickshank *et al.,* 1975).

2.2.1.3.3 Voges- Proskauer test:

It is based upon the production of acetyl methyl carbinol as a product of dextrose metabolism by certain bacteria. This substance is readily oxidized by atmospheric oxygen, in alkaline medium, giving diacetyl

which, in turn, reacts with the amino acid arginine in the medium to give a pink colour when alpha naphthol is added (Cruickshank *et al.,* 1975)

2.2.1.3.4 Citrate utilization test:

It is based on the ability of some organisms to utilize citrate as the sole carbon and energy source for growth, and an ammonium salt as the sole source of nitrogen. Inoculation of Simmon's citrate medium (modification of Koser's citrate medium with agar and an indicator) with a 24 hours culture of the tested organism and incubation at 37^{o}C for 2-3 days. Growth and a change of colour of the indicator from light green to blue, due to alkaline reaction following citrate utilization (Collee *et al*., 1996)

2.2.1.3.5 Indole production test:

It is based on the ability of certain bacteria to decompose the amino acid tryptophane to indole. By inoculation of peptone water with the test organism and incubation for 2 days at 37 °C and addition of Kovac's reagent (ρ-dimethyl-aminobenzaldehyde), a red colour indicates the presence of indole (Collee *et al*., 1996)

2.2.1.3.6 Hydrogen sulphide production test:

It is based upon the ability of some bacteria to produce H_2S from sulphur containing amino acids by reduction. H_2S may be tested by suspending strip of filter paper impregnated with lead acetate above the culture, H_2S is demonstrated by its ability to form black insoluble ferrous sulfide after incubation of the inoculated peptone water at 37ºC for 2-3 days (Collee *et al*.,1996)

2.2.1.3.7Catalase test:

This demonstrates the presence of catalase , an enzyme that catalyses the release of oxygen from hydrogen peroxide.One dropl of hydrogen peroxide solution was poured over a 24 h nutrient agar slope culture of the test organism and the tube is held in slanting position.The production of gas bubbles from the surface of the solid culture material indicates a positive reaction. (Collee *et al*., 1996)

2.2.1.3.8. Coagulase test:

It is based on the presence of the enzyme coagulase in the cell of some bacteria. In a test tube, 1 ml of a l in 10 dilution of sterile citrated human plasma in saline is added to few drops of a 24 hrs. Inoculated broth culture of the test organism, and the mixture incubated at 37 °C and

examined for coagulation after 1, 3, and 6 hrs. The formation of a clearly visible clot indicates a positive coagulase test**.** (Harris, 1964).

2.2.1.3.9. Oxidase test:

It is based upon the presence of the enzyme oxidase in the cells of certain bacteria. The oxidase enzyme catalyzes the transport of electrons between electron donors in the bacteria and a redox dye- tetra methyl, para-phenylene diamine, a freshly prepared oxidase reagent when added in a solid growth media, rapidly develops a purple colour at the colonies of oxidase- positive organism (Cruickshank *et al.,* 1975; Salle, 1961).

2.2.1.3.10 Urease test:

It is based upon the presence of enzyme urease in the cells of certain bacteria. The test organism cultured in a medium which contains urea and the indicator phenol red. When the organism is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the realese of ammonia, the medium becomes alkaline and the indicator colour change from yellow to pink (Cheesbrough, 1996)

2.2.1.3.11. Deoxyribonucleic (DNase) test:

Staphylococcus aureus produces the enzyme deoxyribonuclease (DNase). The test organism was cultured on a medium containing (DNA). After over night incubation the colonies were tested for DNase production by flooding the plate with a (1N) HCl acid solution, the acid precipitated unhydrolysed DNA. DNase producing colonies were therefore surrounded by clear areas indicating DNA hydrolysis (Cheesbrough, 1996).

2.2.2. Plant materials

The two medicinal plants used in this study were collected from different parts of Sudan, by herbalists in collaboration with the Institute of Traditional Medicine, during 2005. They were authenticated by the researcher Haider Abdelgadir and Wail Elsadig Abdalla, Medicinal and Aromatic Plants Research Institute (MAPRI). Voucher specimens were deposited by the herbarium of the Institute.

Data concerning the description of the habitat, the local names, traditional methods of preparing the herbal preparations, the way of application and the diseases they treat were obtained from the local herbalists and recorded.50 grams of each plant sample were powdered by grinder and

extracted, as described below in the experimental section and then subjected to antimicrobial activity screening.

Plants screened are listed in Table (6) with their botanical names, synonyms, families, morphological part used, place of collection, vernacular names and folkloric uses.

2.2.2.1. Preparation of crude extracts:

Each of the coarsely powdered plant material (50 g) was exhaustively extracted for 20 hours with chloroform in Soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure using Rota-vap. The extracted plant material was then air-dried, repacked in the Soxhlet and exhaustively extracted with methanol. The methanolic extract was filtered and evaporated under reduced pressure again using Rota-vap.Each residue was weighed and the yield percentage was determined. The chloroform residue (2 g) was dissolved or suspended in a mixture containing methanol: petroleum ether (2:1) to a final volume 20ml (con. 100 mg/ml). The methanol residue (2g) was dissolved in methanol 20 ml (con. 100mg/ml), and kept in refrigerator until used.

For aqueous extract 100 g of each plant sample was soacked with 500 ml hot water for 4 hours then filtered with Whattman filter paper. Extracts kept in deep freezer for 48 hours, then induced in freeze dryer till

completely dried. The residue was weighed and the yield percentage was determined. The aqueous residue (2g) was dissolved in sterile distilled water 20 ml (con. 100mg/ml), and kept in refrigerator until used.

2.2.3. Preparation of the test organisms:

2.2.3.1 Preparation of bacterial suspensions:

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37º C for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8- 10^9 C.F.U/ ml. The suspension was stored in the refrigerator at 4° C till used.

The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Misra, 1938). Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the

stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

2.2.4 *In vitro* testing of extracts for antimicrobial activity:-

2.2.4.1 Testing for antibacterial Activity:

The cup-plate agar diffusion method **(**Kavanagh, 1972**)** was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts.

One ml of the standardized bacterial stock suspension 10^8 –10^9 C.F.U/ ml were thoroughly mixed with 100ml of molten sterile nutrient agar which was maintained at 45 ºC. 20ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes.

The agar was left to set and in each of these plates 4 cups (10 mm in diameter) was cut using a sterile cork borer (No. 4) and agar discs were removed.

Alternate cups were filled with 0.1 ml sample of each of the 4 extracts in methanol using automatic microlitre pipette, and allowed to

diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for each extract against each of the test organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

2.2.4.2. Testing the susceptibility of clinical isolate to extracts:

Using the standard cup plate agar diffusion technique, the clinical strains were examined for susceptibility to the extracts, which showed activity against standard bacterial organisms.

2.2.5. Determination of minimum inhibitory concentration (MIC) by agar plate dilution method:

The principle of the agar plate dilution is the inhibition of growth on the surface of the agar by the plant extracts incorporated into the medium.

Plates were prepared in the series of increasing concentrations of the plant extract. The bottom of each plate was marked off into 6 segments. The organisms tested were grown in broth over night to contain 10^8 organisms per ml.

A loop-full of diluted culture is spotted with a calibrated loop that delivers 0.001 ml on the surface of each segment. The end point of

MIC is the least concentration of antimicrobial agent that completely inhibits the growth. Results are reported as the MIC in mg/ml of crude extract.

2.2.6. Wound healing activity of *Kigelia africana:-*

The wound evaluation model (Arzi *et al.,* 2003**)** was adopted with some minor modification to assess the wound healing activity of a selected plant extract.

2.2.7.1. Ointment preparation

Polyethylene glycol was used as a water soluble base to prepare ointments of *Kigelia africana* extracts in 1, 2, 5% concentrations

Polyethylene glycol used (1:1) mixture of 200: 2000 PEGs, The mixtures were stirred gently by infusion in waterbath till they are homogenously distributed and then cooled with continuous stirring.

2.2.7.2. Experimental animals:

Swiss Wistar Albino rats of either sex, weighing 80-100g were used. Animals were supplied by the National Experimental Animal House (NEAH), Medicinal and Aromatic Research Institute (MAPRI), National Center for Research (NCR), Ministry of Science and Technology (MOST), Sudan.

The rats were housed individually in a ventilated Animal house before and after surgery. They had access to standard diet which has been prepared in National Experimental Animal House (NEAH) supplemented with water adlibitum (as much as one likes).The holding room was illuminated with 12 hours.Light/dark cycles. Room temperature was between 30-35 C^{o} with 45% to 55% humidity.

2.2.7.3. *In vivo* wound healing activity of *Kigelia africana* extracts (non-infected rats):

Full thickness wounds were made in the skin of the tested animals according to the model of (Arzi *et al*., 2003).

Hair of the lower back and right flank of animals was fully shaved. Rats were lightly anaesthetized by inhalation using Halothane.

The animals were held in standard crouching position, and the mobile skin of flank was gently stretched and held by fingers. A metal circular object measuring 1 cm in diameter was placed on stretched skin and an outline of the object was traced on the skin using a fine tipped pen.

The wound was made by excising the skin within the border of the object to level of loose subcutaneous tissue, using sterile forceps and scalpel blade

The artificial wounds were circular with a diameter of 1 cm.

The first day of the experiment was regarded as day Zero.

Animals were divided into five groups, each containing five animals:

Group 1 (wound only):

Untreated control group, wounds were left without treatment.

Group 2 (wound + Fucidin ointment):

Wounds of these animals were treated topically with fucidin ointment every 24 hours as standard healing agent starting from first day.

Group 3 (wound+ *Kigelia africana* ointment in polyethylineglycol):

Wounds of these animals were treated topically with *Kigelia africana* ointment every 24 hours starting from first day.

2.2.7.4. Evaluation method of wound healing percentage:

In order to determine the rate of wound healing, every 24 hours, each animal was held in the standard crouching position and two diameters of the wound circle (horizontal and vertical) were measured using a transparent ruler. Measurement errors were minimized by

repeating each measurement three times at the same moment and using an average of the calculations.

The area of the wound in day zero was considered as 100% and the wound areas on subsequent days were compared with the wound on day zero.

Healing percentage in a certain day was the difference between the initial wound (in zero days) and healing wound on that certain day.

3. RESULTS

3.1 Isolations and Identification of Clinical Isolates:

3.1.1 Identification of *Eschericha coli*

3.1.1.1 On MacConkey agar medium, red colonies were observed as a result of lactose fermentation.

3.1.1.2 Microscopical examination:

With Gram staining technique, Gram-negative rods with no special arrangement were seen.

3.1.1.3 Biochemical reactions:

The biochemical properties of the isolates are summarized in Table 5.

All isolates fermented lactose, mannitol and glucose with production of acid and gas. All of them fermented sucrose with acid production and gas formation.

All of the isolates were methyl red positive and Voges Proskauer negative. All isolates gave indole positive result. None of the isolates produced urease, utilize citrate or gave positive oxidase.

All isolates did not change the yellow colour of K.I.A. both in slant and butt, with the absence of H_2S production, but all isolates produced gas.

All these led to identifying these clinical isolates as *Escherichia coli*. Table (5).

3.1.2 Identification of *Proteus vulgaris:*

Cultural characteristics:

On nutrient agar, fishy smell and swarming appearance was clear. On MacConkey agar medium, pale coloured colonies were observed as a sign of non-lactose fermentation**.**

Microscopical examination:

With Gram staining technique, Gram-negative rods with no special arrangement were seen

Biochemical reactions:

All isolates were non-lactose and non-mannitol fermenters, but were sucrose fermenters.

Most of them did not ferment sucrose with acid production and some formed gas. Most of them fermented glucose; methyl red positive and all was Voges Proskaur negative.

All of them were oxidase negative, most utilized citrate and produced urease, and most were indole negative.

All isolates changed the colour of slope of KIA from yellow to red and maintained the yellow colour of butt. Most were H_2S producers and gas non-producers.

All these led to identifying these clinical isolates as *Proteus vulgaris.* (Table 5).

3.1.3. Identification of *Pseudomonas aeruginosa*

Cultural characteristics:

On nutrient agar, most of the isolates produced blue-green pigments, which diffused in the surrounding medium.

Microscopical examination:

With Gram staining technique, Gram-negative rods with no arrangement were seen.

Biochemical reactions:

All isolates were non-lactose and non-sucrose fermenters, but glucose fermenters.

Most of them were methyl red negative and all were Voges Proskaur negative. Most were oxidase positive, indole negative, did not utilize citrate and did not produce urease.

Regarding KIA, all of them changed the slope colour to red, and most changed the butt to red, without H_2S or gas production.

All these led to identifying these clinical isolates as *Pseudomonas aeruginosa*.Table (5).

3.1.4**. Identification of *Staphylococcus aureus (S.aureus)***

Cultural characteristics:

On nutrient agar, golden yellow colonies were observed. On mannitol salt agar, it changed the colour of medium from red to yellow.

Microscopical examination:

With Gram staining technique, Gram-positive cocci arranged in grape like clusters were seen.

Biochemical reactions:

All the 25 *S. aureus* strains were isolated from wound and abscess fermented lactose with production of acid and did not form gas. All of them fermented sucrose and mannitol with acid production. All of them fermented glucose with either acid or acid and gas production.

All of them were catalase positive, coagulase positive, most were DNase positive.

All these led to identifying these clinical isolates *as Staphylococcus aureus.*

Table (5). Biochemical tests used for the identification of clinical isolates

Type &no of isolates	Suc	Lact.	Mann.	Gluc.	M.R.	V.P	Cit.	Ind.	Oxid	Ur.	K. I. A slant
E.col i(*25)*	+	+	+	+	+	-	-	+	-	-	Y
Pr.vulgaris (10)	+	-	-	+	+	-	+	-	-	+	R
Pr.vulgaris 15)	+	-	-	+	+	-	+	-	-	+	R
P.*aeruginosa* (13)	+	-	-	+	+	-	+	-	-	+	R
P.*aeruginosa* **(7)**	+	-	-	+	+	-	+	-	-	+	R
P.*aeruginosa* (5)	+	-	-	+	+	-	+	-	-	+	R

Suc=sucrose. Lact= lactose. Mann=mannitol. Gluc= glucose.M.R=methyl red. V.P=voges proskauer.Cit=citrat Ind=indol. Oxid=oxidase. Ur=urease

3.2 Screening of antibacterial activity of *Guiera senegalensis* & *Kigelia africana*:

In the preliminary screening for antibacterial activity of two Sudanese medicinal plants, belonging to two families, the total number of extracts examined against the five tested standard organisms was 6. Of these extracts, 5 exhibited inhibitory activity against the five tested standard bacteria. The other extract was devoid of any activity.

All methanolic and aqueous extracts of the two medicinal plants exhibited innhibitory effects against all the tested standard organisms.

All chloroformic extracts of the two medicinal plants exhibited low inhibitory effects against the tested standard organisms Table (6).

Interpretation of results:

The means of the diameters of the growth inhibition zones obtained in the experiments were shown in Table (6) and the results were interpreted susceptible, intermediate and resistant Table (7).

On the basis of the results obtained with standard chemotherapeutic agents against the same standard tested microorganisms Table (8) plant extracts resulting in more than 18 mm growth inhibition zones are considered to possess relatively high antibacterial activity, and those resulting in 14-18 mm inhibition zones are of intermediate activity, and those resulting in zones below 14 mm were considered of low activity. (Cruickshank *et al*., 1975).

Five plant extracts exhibited inhibitory activity against *Staphylococcus aureus* Table (9) and Figure (1).

Four plant extracts exhibited inhibitory activity against *Bacillus subtilis* Table (10).

Escherichia coli were inhibited by four-plant extracts. Table (11).

Four plant extracts were found to be effective against P*roteus vulgaris* Table (12).

Pseudomonas aeruginosa was inhibited by five plant extracts. Table (13).

3.3 Screening of antifungal activity of *Guiera senegalensis* & *Kigelia africana*:-

In this study, the two medicinal plant extracts were also screened for their antifungal activity against two fungi *Aspergillus niger* and *Candida albicans.* Table (14).

The antifungal activities of these two plant extracts were compared with those of Nystatin and Clotrimazole as reference antifungal agents. Table (15**) (**Cruickshank *et al.,* 1975).

Out of the 6 extracts screened, 4 (66, 7%) exhibited inhibitory activity against the tested fungi. The other 2 (33, 3%) extracts were devoid of any activity against the two-tested fungi.

Methanolic and aqueous extracts of the two plants exhibited antifungal activity against the two tested fungi.

Chloroformic extracts of the two medicinal plant extracts did not exhibit antifungal activity against any of the tested fungi.

Interpretation of results: -

According to the interpretation of the results in terms of susceptible, moderate and inactive it had been found that four extracts (66, 7%) were moderate and two extracts (33, 3) were inactive.

Four plant extracts exhibited inhibitory effect against *Candida albicans,* Table (16). *Aspergilus niger* inhibited by four plant extracts. Table (17).

3.4 Determination of the minimum inhibitory concentrations (MICs):

The minimum inhibitory concentrations of the most active extracts (i.e. the methanolic and aqueous extract of each plant) were determined against the standard organisms (*Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans.*

The results were summarized inTable (18) as mg/ml of crude extract.

Aspergillus niger and *Candida albicans* were the most resistant (less susceptible) organisms.

3.5 Susceptibility of the clinical isolates to selected plant extracts exhibiting high antibacterial activity: -

Depending on the results of testing 6 plants extracts against the standard bacteria,the most active plant extracts were tested against 100 clinical isolates (any inactive plant extract against standard bacteria, was excluded from being tested against the clinical isolates).

Aqueous leaves extract of *Guiera sengalensis* exhibited high inhibitory activity against all clinical isolates, while methanolic leaves extract of the plant exhibited inhibitory activity against 97% of the clinical isolates. Table (19) and Figure (2).

Methanolic fruit extract of *Kigelia africana* exhibited high inhibitory activity against all clinical isolates, while aqueous extract of the plant

exhibited activity against 93% of the clinical isolates.Ttable (20) and Figure (3).

Two *Staphylococcus aureus* clinical isolates were resistant in their activity to the medicinal plant extracts.Table (21).

Four *Escherichia coli* clinical isolates showed resistance to the two plant extracts. Table (22).

Two isolates of *Pseudomonas aeruginosa* were found to be resistant to the two medicinal plant extracts.Table (23).

Two *Proteus vulgaris* strains were not susceptible to the two plant extracts.Table (24).

3.6 Wound healing activity of Kigelia africana:-

Interpretation of the results:-

In the first (untreated wounded) group, healing was completed in 11 days. In the group (wounded /treated with fucidin ointment) more than 11 days were required for the completion of healing. In the third group (wouded /treated with PEG containing *Kigelia africana* 2% ointment) the healing period was reduced to 10 days in three rats. Figure (4) .The healing rate in the other two rats was higher than the healing rate of the rats of the other two groups on the same day.Table (25) and Figure (5).

Sign of inflammation (pus, exudates, black debris redness and pictured) were not observed on the rats treated with *Kigelia africana* ointment.

These signs observed until the last of the experiment on the untreated and fucidin treated groups.

Significant differences between PEG containing *Kigelia africana* 2% ointment and fucidin ointment groups were observed at the first day of the treatment until the end of the treatment course.Significant differences between groups was calculated.

Table (6) Preliminary screening for antimicrobial activity of *Kigelia africana and Guiera senegalensis* plant extracts:-

of on	Folkloric use (local)	Part used (extracted	Solvent system	Yield %	Test organism usd */MDIZ mm**						
					B.s	***S.a***	***E.c***	***Pr.v***	***P.a***	***A.nig***	***C.al***
ım	Wound and Abscesses	Fruits	$CHCL_3$	3.2	12	14	13	-	11	-	-
			MeOH	18.4	25	18	19	22	18	15	15
			H_2O	6.2	16	24	21	18	22	17	16
	Antipyretic,	Leaves	$CHCL_3$	.65	12	11	-	12	14	-	12
ıins	Antidiabetic		MeOH	20.2	29	23	20	20	22	18	18
			H_2O	3.5	20	28	27	22	24	16	17

B.s= Bacillus subtilis, S.a =Staph aureus, E.c=Escherichia coli, Pr.v=Proteus vulgaris, P.a=Pseudomonas aeruginosa,A.nig= Aspergillus niger, C.al= Candida albicans .

M.D.I.Z.=Mean diameter of growth inhibition zone in (mm),Average of 2 replicates concentration used= 100mg/ml of 0.1 ml/cup.

- = No activity**.**

Table 7: Susceptibility of standard Organisms to *Kigelia africana and Guiera senegalensis* plant extracts:-

Organisms	NO. of extracts *		
	Active **	**Moderate*****	**Low******
Bacillus subtilis	3	1	2
S. aureus	3	2	1
Escherichia coli	4	-	2
Proteus vulgaris	3	1	2
Pseudomonas aeruginosa	3	2	1

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean dimeter of growth inhibition zone in

Table 8: Antibacterial Activity of reference drugs against standard organisms

Drug	Concentration used µg /ml	*Standard organisms used /**MDIZ (mm)				
		B.s	***S.a***	***E.c***	***Pr.v***	***Ps.a****

Ampicillin	40	16	-	-	20	-
	20	14	-	-	18	-
	10	13	-	-	16	-
	5	12	-	-	-	-
Benzyl penicillin	40	-	38	-	-	-
	20	-	33	-	-	-
	10	-	28	-	-	-
	5	-	24	-	-	-
Cloxacillin	40	-	29	-	-	-
	20	-	27	-	-	-
	10	-	22	-	-	-
	5	-	18	-	-	-
Gentamcin	40	30	20	22	20	18
	20	20	16	18	16	16
	10	16	14	15	14	12
	5	16	12	11	-	-

Key:*Standard organisms tested: *B.s. = Bacillus subtilis*,

S.a. = Staphylococcus aureus, E.c. = Escherichia coli, *Pr.v. = Proteus vulgaris*, *Ps.a. = Pseudomonas aeruginosa*.

**MDIZ : Mean diameter of growth inhibition zone in (mm)

Interpretation of results

MIZD (mm): >18 mm : Active

: 14 – 18 mm: Moderate

: < 14 mm

-: No inhibition zone

Table 9: Susceptibility of *Staphylococcus* aureus to *Kigelia africana and Guiera senegalensis* plant extracts:-

Solvent (extracts)	No. of extracts *		
	Active**	**Moderate*****	**Low******
$CHCL_3$	-	1	1
MeOH	1	1	-
H_2O	2	-	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z. = 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean diameter of growth inhibition zone in (mm)

Table 10: Susceptibilty of *Bacillus subtilis* to *Kigelia africana and Guiera senegalensis* plant extracts:-

Solvent (extracts)	No. of extracts*

	Active**	**Moderate*****	**Low******
$CHCL_3$	-	-	2
MeOH	2	-	-
H_2O	1	1	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean diameter of growth inhibition zone in (mm)

Table 11: Susceptibilty *of Escherichia coli* to *Kigelia africana and Guiera senegalensis* plant extracts:

Solvent (extracts)	No. of extracts *		
	Active**	**Moderate****	**Low********
$CHCL_3$	-	-	2
MeOH	2	-	-
H_2O	2	-	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm
*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean diameter of growth inhibition zone in (mm)

Table 12: Susceptibilty of *Proteus vulagris* to *Kigelia africana and Guiera senegalensis* plant extracts:

Solvent (extracts)	No. of extracts*		
	Active**	Moderate**	Low ****
$CHCL_3$	-	-	2
MeOH	2	-	-
H_2O	1	1	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean diameter of growth inhibition zone in (mm)

Table 13: Susceptibilty of *Pseudomonas aeruginosa* to *Kigelia africana and Guiera senegalensis* plant extracts:-

Solvent (extracts)	No. of extracts*		
	Active**	Moderate***	Low*****
$CHCL_3$	-	1	1
MeOH	1	1	-
H_2O	2	-	-

* No. of extracts = 6
** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean dimeter of growth inhibition zone in (mm)

Table 14: Susceptibility of standard fungi to *Kigelia africana and Guiera senegalensis* plant extracts:-

Organisms	**No. of extrcts***		
	Active**	**Moderate*****	**Low********
Aspergillus niger	-	4	2
Candida albicans	-	4	2

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

****Low = M.D.I.Z. <14

-=No inhibition zone

M.D.I.Z. = Mean dimeter of growth inhibition zone in (mm)

Table 15: Antifungal activity of reference drugs used against the

standard organisms.

Drug	**Concentration used(µg /ml)**	**MDIZ *(mm)**	
		Aspergillus niger	***Candida albicans***
Clotrimazole	20 10 5	24 19 16	43 33 30
Nystatin	50 25 12.5	17 14 -	28 28 23

MDIZ * : Mean diameter of growth inhibition zone (mm)

Interpretation of results

MDIZ (mm): > 18 mm : Active

: 14 – 18 mm: Moderate

:< 14 mm : Low

- : No inhibition zone

Table 16: Susceptibility *of Candida albicans* to *Kigelia africana and Guiera senegalensis* plant extracts:-

Solvent (extracts)	**No. of extracts***		
	Active**	**Moderate*****	**Low********
$CHCL_3$	-	-	2
MeOH	-	2	-
H_2O	-	2	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z. = 14-18 mm

**** Low = M.D.I.Z. <14

- : No inhibition zone

M.D.I.Z. = Mean dimeter of growth inhibition zone in (mm)

Table 17: Susceptibility of *Aspergilus niger* to *Kigelia africana and Guiera senegalensis* plant extracts: -

Solvent (extracts)	**No. of extracts***		
	Active**	**Modrate*****	**Low********
$CHCL_3$	-	-	2
MeOH	-	2	-
H_2O	-	2	-

* No. of extracts = 6

** Active= M.D.I.Z. >18 mm

*** Moderately active = M.D.I.Z.= 14-18 mm

**** Low = M.D.I.Z. <14

- : No inhibition zone

M.D.I.Z. = Mean diameter of growth inhibition zone in (mm

Table18: Determination of the minimum inhibitory concentration. (MIC)(mg/ml) of crude extract against standard organisms

Plant	**Part**	**Solvent**	***B.s***	***S.a***	***E.c***	***P.v***	***Ps.a***	***A.nig***	***C.a***
G. se	Leaves	MeOH	>75	9.38	>75	>75	37.5	>100	>100
Ki. a	Fruits	MeOH	37.5	37.5	75	75	75	>100	>100

B.s=Bacillus subtilis. S.a= Staphylococcus aureus. E.c= Escherichia coli. P.v= Protus vulgaris. Ps.a= Pseudomonas aeruginosa. A.nig= Aspergillus niger. C.a= Candida albicans.

G. se= Guiera senegalensis; Ki.a = Kigelia Africana

Table 19: The activity *of Guiera senegalensis* leaves against 100 clinical isolates

Organism tested	Solvent	No. of clinical isolates			
		Susceptible	**Intermediate**	**Resistant**	**Total**
E. coli	MeOH	18	6	1	25
	H_2O	22	3	-	
Pr. Vulgaris	MeOH	17	8	-	25
	H_2O	24	1	-	
Ps.aeruginosa	MeOH	22	1	2	25
	H_2O	24	1	-	
S. aureus	MeOH	24	1	-	25
	H_2O	22	3	-	
Total					100

Table 20: The activity of *Kigelia africana* fruits against clinical isolates

Organism tested	Solvent	No. of clinical isolates			
		Sensitive	**Intermediate**	**Resistant**	**Total**
E. coli	MeOH	22	3	-	
	H_2O	19	3	3	25
Pr. Vulgaris	MeOH	24	1	-	
	H_2O	14	9	2	25
Ps.aeruginosa	MeOH	25	-	-	
	H_2O	11	14	-	25
S. aureus	MeOH	21	4	-	
	H_2O	18	5	2	25
Total					100

Table 21: Susceptibility of S*taphylococcus aureus* clinical isolates against selected plant extracts exhibiting high antibacterial activity

		ameter of inhibition zones,in(mm)																				
		5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
		24	25	24	25	26	22	22	25	25	20	25	24	22	19	22	19	24	20	20	21	18
		20	22	23	25	24	21	29	26	24	20	25	22	25	18	20	23	20	18	18	20	25
		16	25	25	20	31	16	24	29	19	22	23	32	21	23	26	15	22	30	24	22	32
		21	21	24	22	25	14	20	19	21	19	20	20	19	15	18	13	12	15	15	19	20

G.se =Guiera senegalensis

Ki.a= Kigelia africana

Table 22: Susceptibility *of Escherichia coli* clinical isolates against selected plant extracts exhibiting high antibacterial activity

		meter of inhibition zones,in (mm)																			
		5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
	)	24	15	24	15	20	20	20	15	20	15	23	20	15	25	19	20	19	20	20	25	25
	;	26	22	30	22	15	20	25	15	20	22	22	22	20	20	20	20	19	21	20	23	15
	;	25	32	32	21	25	20	15	19	25	20	30	25	24	24	15	30	15	25	25	25	25
	!	22	23	16	11	11	15	22	20	20	15	0	23	24	21	20	21	20	20	21	20	20

G.se =Guiera senegalensis

Ki.a= Kigelia africana

Table 23: Susceptibility of *Pseudomonas aeruginosa* clinical isolates against selected plant extracts exhibiting high antibacterial activity

	ameter of inhibition zones,in(mm)																				
		5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
	)	15	25	20	20	19	20	26	20	25	24	20	20	10	22	20	23	30	29	20	25	11
	;	20	25	20	19	20	23	20	22	20	22	20	19	25	20	25	22	20	27	22	25	22
	:	20	31	25	30	22	25	28	21	25	20	23	25	20	30	26	21	25	24	24	30	25
	)	18	20	18	18	19	18	16	19	18	18	18	22	18	20	19	18	20	22	18	18	23

G.se =Guiera senegalensis

Ki.a= Kigelia africana

Table 24: Susceptibility of *Proteus vulgaris* clinical isolates against selected plant extracts exhibiting high antibacterial activity

		meter of inhibition zones, in mm																			
		5	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
	)	20	23	24	19	20	17	22	20	18	20	20	15	18	19	17	20	17	25	23	22	20
	:	22	22	25	20	22	20	25	15	22	25	25	22	21	25	22	25	27	25	22	24	23
	;	23	19	32	25	24	26	27	20	20	31	24	15	22	26	24	27	29	32	24	27	28
	+	19	18	16	17	18	19	18	19	22	18	22	24	11	18	20	22	22	19	16	15	18

G.se =Guiera senegalensis Ki.a= Kigelia africana

Table 25: Percentage of wound healing activity *of Kigelia africana* plant extract on five Albino rats

		Group 2(wound + Fucidin)					Group 3(wound +K.A.2%PEG oint				
	Rat5	**Rat1**	**Rat2**	**Rat3**	**Rat4**	**Rat5**	**Rat1**	**Rat2**	**Rat3**	**Rat4**	**Rat5**
	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	225	16%	27%	0%	15%	20%	28%	27%	33%	33%	40%
	50%	35%	37%	24%	43%	43%	57%	445	55%	56%	58%
	54%	54%	50%	42%	43%	49%	57%	47%	58%	56%	68%
	61%	62%	54%	29%	54%	53%	58%	54%	69%	62%	69%
	61%	64%	54%	29%	58%	54%	58%	50%	72%	65%	75%
	65%	70%	64%	39%	65%	60%	68%	60%	73%	74%	75%
	75%	76%	77%	50%	72%	69%	68%	74%	75%	74%	76%
	90%	81%	84%	70%	73%	78%	82%	87%	89%	89%	86%
	93%	89%	94%	85%	93%	89%	90%	97%	98%	100%	100%
	93%	90%	94%	91%	100%	95%	98%	100%	98%	100%	100%
	100%	100%	94%	91%	100%	100%	100%	100%	100%	100%	100%

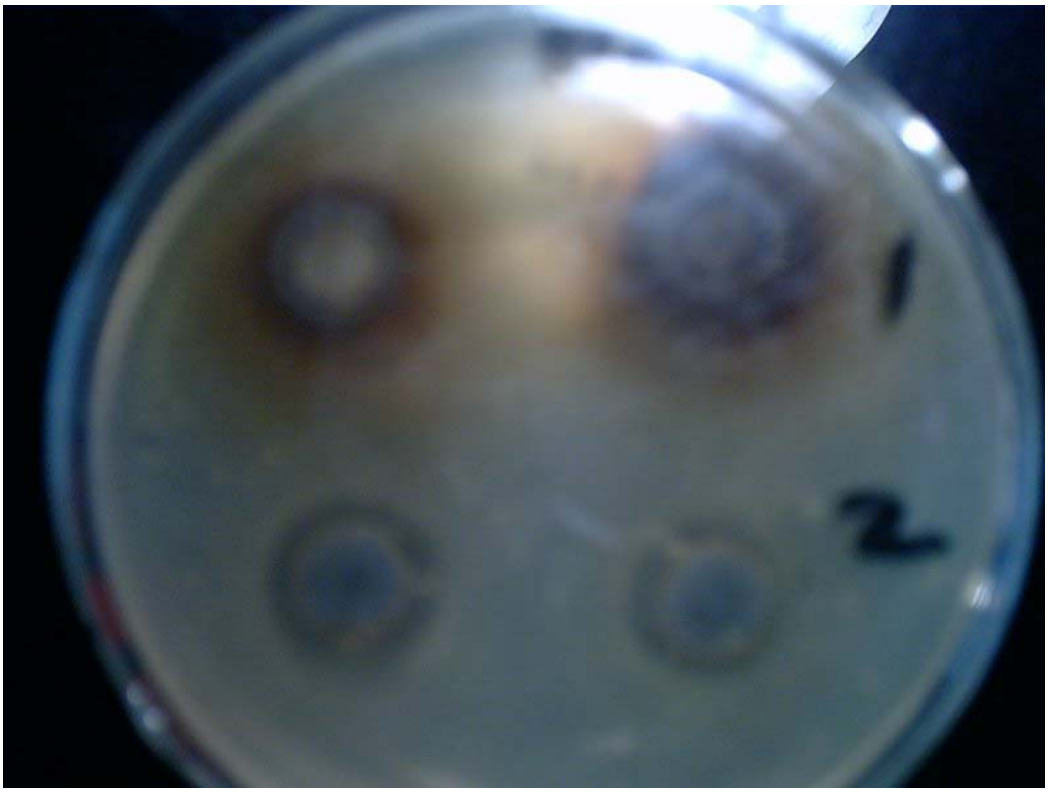
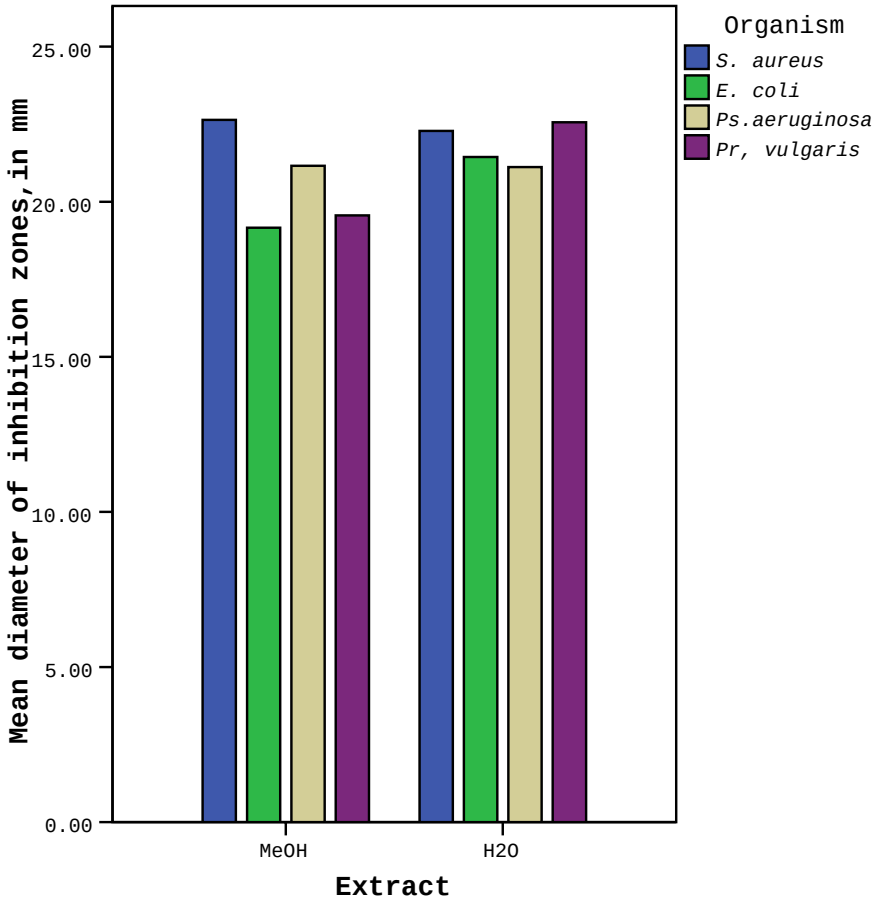


Figure (1)

Antimicrobial activity of *Kigelia africana* (upper) and *Guiera senegalensis* (lower) on S*taphylococcus aureus*



Fgure (2)

Antimicrobial activity of *Guirea senegalensis* leaves extracts against clinical isolates

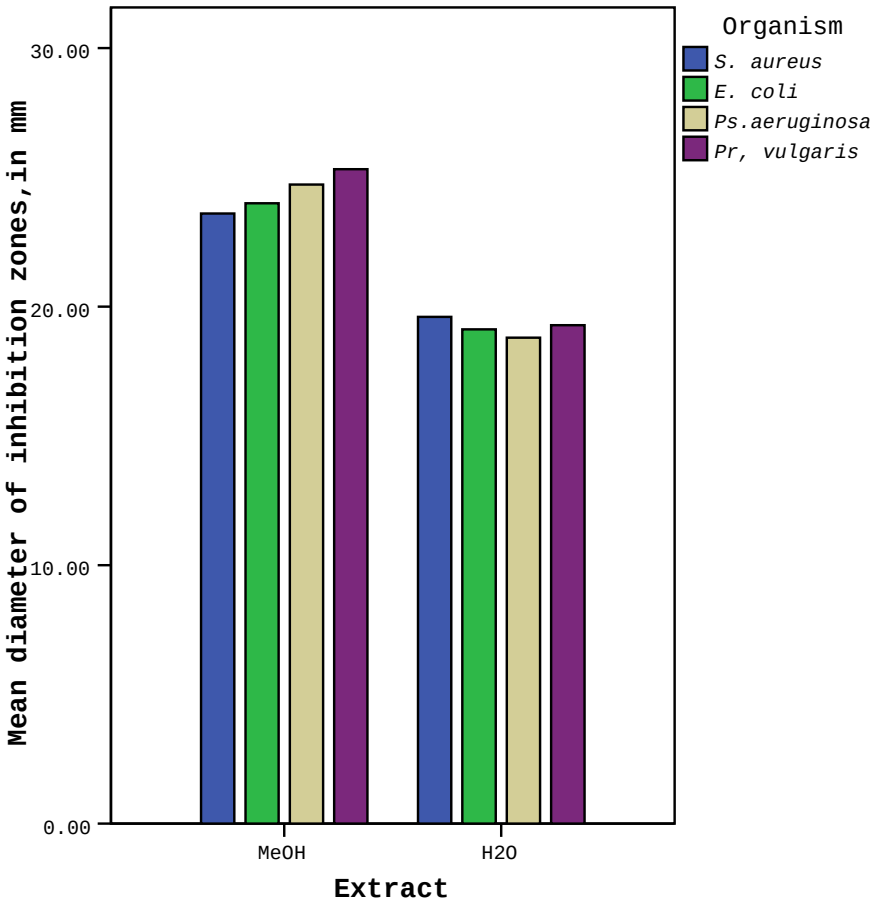


Figure (3)

Antimicrobial activity of *Kigelia africana* fruits extracts against clinical isolates

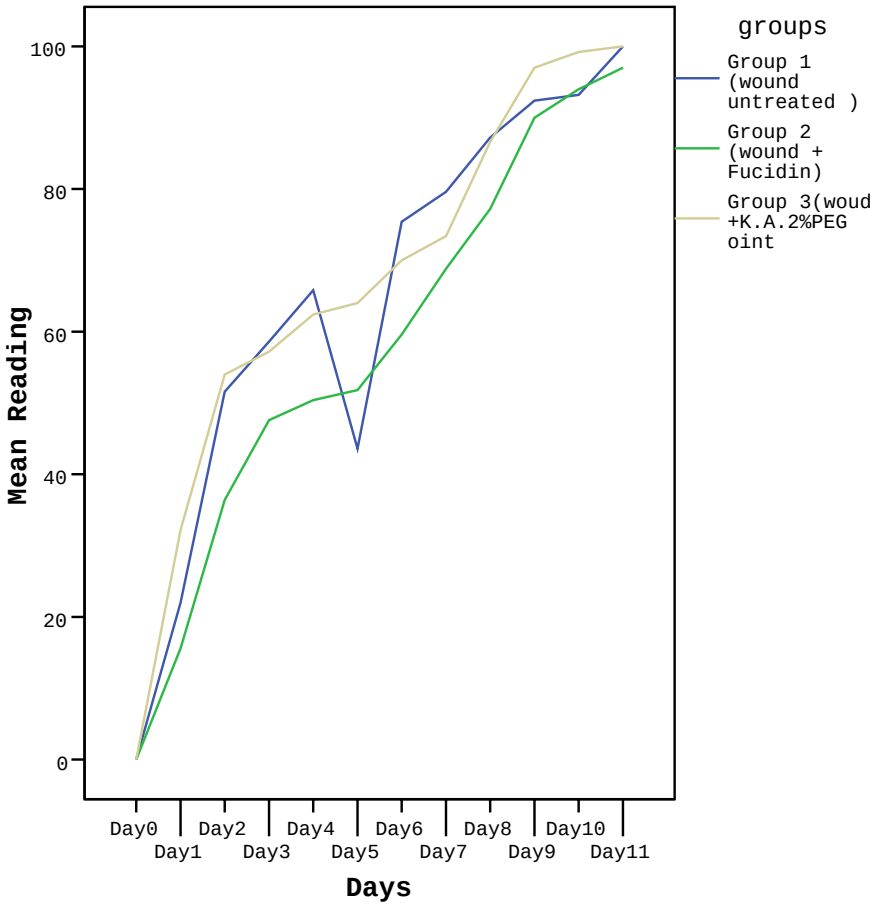


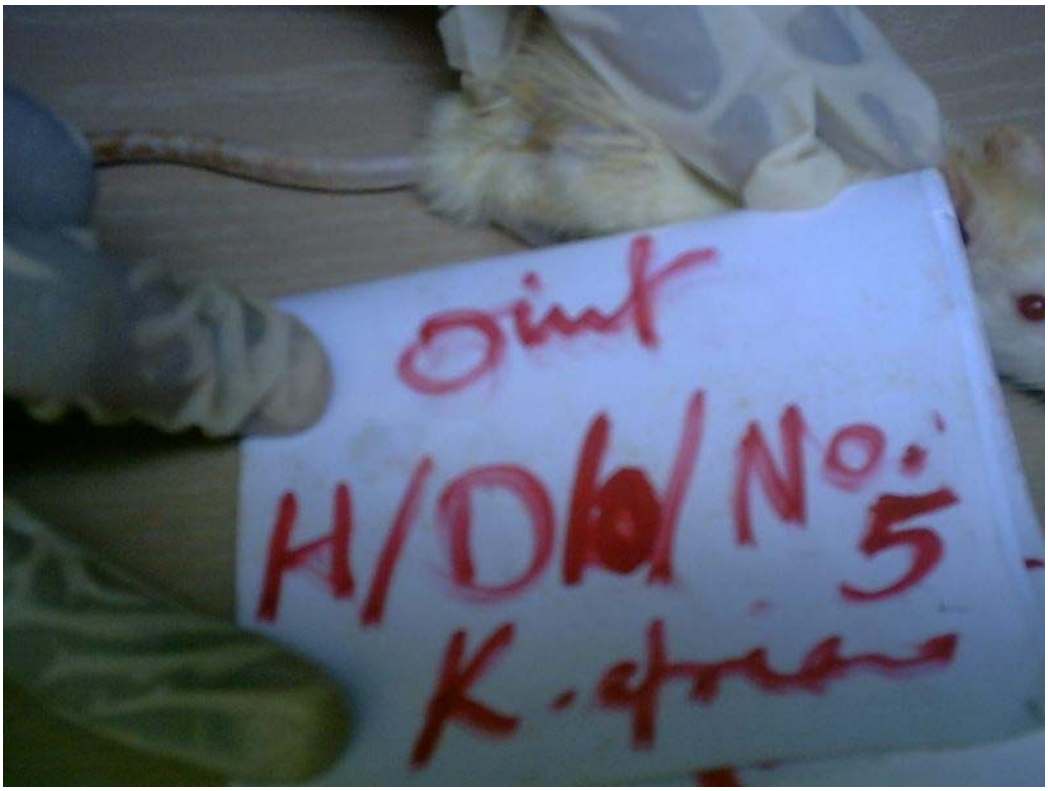
Figure (4)

Percentage wound healing activity of *Kigelia Africa*na

Figure (5)

Wound healing activity of *Kigelia africana* ointment (day 3)

Wound healing activity of *Kigelia africana* ointment (day 6)



Wound healing activity of *Kigelia africana* ointment (day 10)

4. Discussion

4.1The antimicrobial activity of the two medicinal plants:

4.1.1 *Kigelia africana*

In this study the chloroform fruit extract of *Kigelia africana* showed low activity (11-13mm) *against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* intermediate activity (14mm) against *Staphylococcus aureus,* and no activity against *Proteus vulgaris, Aspergillus niger and Candida albicans.*

The methanol fruit extract of *Kigelia africana* exhibited pronounced activity (25mm) against *B.subtilis*, (22mm) against *Proteus vulgaris*, high activity (19mm) against *Escherichia coli* and intermediate activity (15-18mm) against *Staphylococcus* aureua*, Pseudomonas aeruginosa* and against both fungi *Aspergillus niger and Candida albicans.*

Its aqueous fruit extract showed pronounced activity(24mm) against *Staphylococcus aureus,* high activity(21-22)against *Escherichia coli* and *Pseudomonas aeruginosa* and intermediate activity(16-18mm) against *Bacillus subtilis Proteus vulgaris,Aspergillus niger* and *Candida albicans*.

This result is different from that reported by Almagboul (1992) who found that the chloroform and the methanol extracts of *Kigelia africana* gave good results while the aqueous extract was inactive. This could be due to the unknown concentrations used.

Grace *etal.* (2002) tested the stem and fruit of *Kigelia africana* aqueous, ethanol and ethyl acetate extracts, which is the most popular source of traditional medicine throughout Africa, for their antibacterial activity using the microtitre assay. All extracts showed significant activity against the tested Gram positive and Gram negative bacteria, thus supporting the traditional use of the plant in the therapy of bacterial infections. These results are similar to our results.

Ijah and Oyebanji,(2003)determined the activity of the crude extracts of the medicinal plants *Kigelia afrcana,Bridelia ferruginea,Trema nitems* and *Drypetes gossweileri* against *Escherichia coli,Staphylococcus aureus*

Pseudomonas aeruginosa,Klebsiella sp. and *Proteus sp*., causing urinary tract infection. The extracts of the medicinal plants inhibited the growth of the bacteria tested. These results are in agreement with our result.

Gram positive and Gram negative bacteria were equal in their susceptibility to the different plant extracts used in this study and this result contrary to that obtained by Abdalla (2004) who proved that the Gram negative organisms were less susceptible to the extracts than the Gram positive bacteria. It is evident that the patterns of inhibition varied with the plant part, the solvent used for extraction and the organisms tested.

The methanol extract of *Kigelia afrcana* exhibited intermediate activity against *Aspergillus niger* which is almost similar 5µg/ml Clotrimazole and 25µg/ml Nystatin i.Its aqueous extract exerted intermediate activity against *Aspergillus niger* which is similar to50µg/ml Nystatin

4.1.2*Gueria senegalensis*

In the present study, the chloroform leaves extracts of *Gueria senegalensis* showed low activity (11-12) against *Bacillus subtilis, Staphylococcus aureus, Proteus vulgar* and *Candida albicans* and an intermediate activity against *Pseudomonas aeruginosa* while no activity against *Escherichia coli* and the fungus *Aspergillus niger.*

Both its methanol and aqueous extracts exhibited pronounced activity (20-29mm) against both Gram positive and Gram negative organisms and intermediate activity (16-18mm) against both fungi. These results are similar to that reported by Grand **(**1989**)** who found that the leaves of *Gueria senegalensis* showed antimicrobial activity against the bacteria and fungi tested. Similarly Sanago *etal*. (1998**)** found that a decoction and methanol extracts of *Gueria senegalensis* exhibited significant activity against all strains of bacteria tested and that is suggested to the use of the same solvent.Our results are different from Bosis *etal*. (1997) who found that the crude extract of the same plant possessed a mild antimicrobial effect only on Gram positive bacteria.

The methanol and aqueous extracts of *Gueria senegalensis* leaves showed high activity against both Gram positive and Gram negative organisms which was more than that produced by40µg/ml Ampicillin.The methanol extract inhibited *Bacillus subtilis, Proteus vulgaris* similar to 40µg/ml of Gentamicin, *Pseudomonas aeruginosa Staphylococcus aureus* higher than 40µg/ml of Gentamicin and inhibited *Escherichia coli* similar to 40µg/ml Gentamicin.

The aqueous extract of the same plant inhibited *Bacillus subtilis* similar to 20µg/ml Gentamicin and inhibited *Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa* higher than 40µg/ml of Gentamicin.

The methnol extract of *Gueria senegalensis* showed intermediate activity against *Aspergillus niger* which is almost similar to 10µg/ml Clotrimazole and 50µg/ml Nystatin .Its aqueous extract exhibited intermediate activity against *Aspergillus niger* which is similar to 5µg/ml Clotrimazole and almost similar to 50µg/ml Nystatin.

4.2 Discussion of wound healing activity of *Kigelia africana*

In the present study the methanolic extract of *Kigelia africana* was able to reduce the time required for wound healing. The results were obtained from the faster contraction of the wound treated with *Kigelia africana* extract in comparison with fucidin ointment or untreated groups.

Kigelia africana ointment in 2% Poly ethylene Glycol was more potent than Fucidin ointment as standard healing agent.The percentage of healing with Polyethylene Glycol contaning *Kigelia africana* 2% ointment was significantly higher than Fucidin group from the first day of treatment until the closure of wounds. This is similar with Arzi *et al*. (2003) who found that the healing effect of *Licorice* cream of 10 % is a potent healing agent even better than phenytoin cream

Owolabi&Omogbai. (2007) evaluated the ethanolic extract of *Kigelia africana* for analgesic property using acetic acid induced mouse writhing and hotplate reaction time and anti-inflammatory activity using the carrageenan induced paw odema and its probable mechanism evaluated in mice and guinea pigs.*Kigelia africana* extract showed a dose dependant significant reduction of the number of writhes (p0.001) with 500mg /kg body weight dose giving the highest reduction. The extract showed significant analgesic and anti-inflammatory activity.

Inhibition of the synthesis of prostaglandins and other inflammatory mediators probable accounts for the analagesic and anti inflammatory.

Asekum *etal*.(2007) analysed the volatile constituents of the oil form the leaves and flowers of *Kigelia africana* from Lagos(Nigeria), isolated by hydrodistllation using GC and GC/MS.The leaf oil was found to contain 25 components, while the flower oil contained nine.These components responsible for the bioactivity of this plant extracts.

Not to my knowledge there is a literature concerning wound healing activity of methanolic extracts of the fruits of *Kigelia africana.*However more studies are reguired to elucidate the extract mechanism of *Kigelia africana* in wound healing modles.

5.1 Conclusions

Sudan has huge resources of plants. Exploitation of these plants represents an important means of obtaining cheap and effective drugs, which can participate in the solution of the health problems in the country.

The results of the present work indicate that there are plants with promising high and broad antimicrobial activity, when compared with standard antimicrobial drugs in current use. These findings verified the claimed bioactivity of these plants and their employment in traditional medicine in Sudan.

The methanolic and aqueous extracts of *Guiera senegalensis* and *Kigelia africana* proved to have significant antimicrobial activity and this justifies their traditional uses.

Methanolic extract of *Kigelia africana* was proved to have wound healing activity, and this justifies its traditional use as a wound healing agent.

Recommendations

Pharmacological, toxicological and clinical studies should be carried out on the selected medicinal plants to assess their safety, therapeutic efficacy and potential for commercial utilization.

Formulation of the active extracts and/ or principles in suitable dosage form, with special reference to *Kigelia africana* fruits which proved to be a potent wound healing agent.

Bio-assay-guided fractionation and purification may lead to isolation of the active compounds. The chemical structures of these compounds can then be elucidated. This can then help in:-

i- The standardization of the active.

ii- The study of the structure activity relationship for the production of compounds with improved characteristics.

iii- The study of pharmacokinetics of the pure active compounds and also it helps in the formulation procedures.

References

Abdrabo, AN; Omer, MEA; Elnima, EI; Shayoub,MAA.; Almagboul AZ; (2005) .Wound healing activity of methanolic extract of *Solenostema argel* in poletyyleneglycol ointment.Omdurman Journal of Pharmceutical Sciences 1(1): 43-54.

Adupa, SL; Udopa, AL; Kulk Arni, DR. (1991). Influence of *Tridax procumbens* on dead space wound healing. Fitoterapia. 62(2): 146-1

Ahmed ,AM ; Gumaa, SA.; Abdelmageed, MAM.; Almagboul, AZ.(2007**).***IN vitro* antimicrobial activity of some Sudanese plants used in traditional medicine. Omdurman Journal of Pharmaceutical Sciences 1(3): 367-378.

Ahmed, IH; Awad, MA; Elmahdi, M.; Gohar, HM.; Ghanem, AM.(1995)**.** The effect of some medicinal plants extracts on wound healing in farm animals .Assiut Veterinary Medicinal Journal. 32(64): 236-244.

Ali, MS.; Yaghmour, RMR; Faidi, YR; Salem, K; Al-Nuri, MA. (1998). Antimicrobial activity of 20 plants used in folkloric medicine in Palestinian area. Journal of Ethnopharmacology, 60(3): 265-271.

Almagboul, AZ. (1992). Antimicrobial and Phytochemical Investigations of *Vernonia amygdalina* and other Sudanese Medicinal Plants. Doctorate thesis, Khartoum, University of Khartoum, Sudan.

Ancolio,C; Azaz,N; Mahiou,V; Ollivier, E; Giorgio,CD; Keita,A; Timon,D.; Palansard,G; Giorgio,DC.(2002). Antimicrobial activity of extracts and alkaloid isolated from six plants used in traditional medicine. Phytotherapy Research.16 (7):646-649**.**

Anil, S; Shukla, NY; Sushil, K; Srivastava, A; Kumars, S. (2000). Recent development in plant derived antimicrobial constituents-a review. Journal of Medicinal and Aromatic Plant Sciences, 2(3): 349-405.

Ansel, Hc; Allen, LV.; Popovich, NG (2000). Pharmaceutical Dosage, 7th edition, Lippinctt Willams and Wilkins (pub), New York

Arzi,A .; Hemmati, AA; Amin, M. (2003). Stimulation of wound healing by licorice in rabbits. Saudi Pharmaceutical Journal. 1(2): 57-60.

Asekun, OT. ; Olusegum, E.; Adebola, O. (2007). The volatile constituents of the leaves and flowers of *Kigelia africana*. Flavour and Fragrance Journal. 22(1) 21-23.

Atindehou, KK. ; Kone, M.; Terreaux, C.; Traore, D.; Hostettmann, K.; Dosso, M. (2002). Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. Phytotherapy Research, 16(5): 497-50

Austin, GW. ; Guenim, MF. ; Hokett, SD. (2001). Effect of nicotine on fibroblast beta-1. J. Perodontol. 72(4) 438-44.

Ayensu, ES. (1978).Medicinal plants of West Africa. Reference publications, Inc. (pub.), New York.

Bagchi, GD.; Amrita, S.; Khanuja, SPS. ; Bansal, Rp. ; Singh, SC.; Sushil,K.(1999). Wide spectrum antibacterial and antifungal activities in the seeds of some coprophilous plants of North Indian Plains. Journal of Ethnopharmacology, 64(1): 69-77.

Balchin , M. (1997). Essential oils and 'aromotherapy': their modern role in healing. Journal of the Royal Society of Health, 117(5): 324-329.

Bandara, BMR. ; Hewage, C M .; Jayamanne, DHLW.; Krunaratne, V.; Pinti, MRM. (1990). Biological activity of some steam distillates from leaves of ten species of rutaceous plants.Journal of the National Science, 18(1): 17-77.

Bhacta, T.; Mukherjee, Pk.; Pal, M.; Saha, BP. (1998): Studies on *in vivo* wound healing activity of *Cassia fistula* leaves in rats. Natural Product Sciences. 4(2): 84-87.

Bodeker,G.; Hughes, AM.; Prendergast, HDV.; Etkin, NL.; Harris, DR.; Houghton, PJ.(1998). Wound healing, traditional treatments and research policy. Plants for food and medicine, 1(6): 345-359.

Bosisio, E.; Mascetti, D.; Verotta, L.; Zani, F.; Mazza, P.; Talbot, M. (1997). *Guiera senegalensis J*.F. Gmelin(Combretaceae): Biological activities and chemical investigation. Phytomedicine, 3(4): 339-348.

Boulos, L. (1983). Medicinal plants of North Africa. Reference Publications, Inc. (Pub). USA.

Bushell, AC. (1989). Medical Bacteriology, first Edition, Oxford University Press, New York, 167-193.

Cheesbrough, M. (1996). Medical Laboratory Manual for Tropical Counteries (Microbiology) 14 Bevills Close, Paddington Cambridge Shire (Pub). England.Vol II.

Cheesbrough, M. (2000). District laboratory Practice for Tropical countries. Part2. University Press. Cambridge.

Cheesbrough, M. (2004). District Laboratory Practice in Tropical Countries, Part 2, Low price edition, Cambridge.

Chhabra, SC.; Uiso, FC. (1991).Antibacterial activity of some Tanzanian plants used in traditional medicine. Fitoterapia, 62(6): 499-503.

Collee,JG.;Fraser,AG.;Marmion,BP.;Simmons,A.;Mackie and McCartiny.(1996).Practical Medical Microbiology.14th edition.Churchill Livingstone . New York.

Collett, DM., Aulton, Me. (1991): Pharmaceutical Practice, First edition, Churchill, Livingstone, London.

Couster, LV.; Pashley, MM .; Tattersall, KJ. (1961). Ointments Pharm.Pharma.3 (1): 620-624.

Cowan, ST.; Stell, KJ. (1970). Manual for the Identification of Medical Bacteria.First edition.Cambridge. University Press.

Cruickshank, R.; Duguid, JP.; marmion, BP.; Swain, RH. (1975). Medical microbiology 12th edition. Churchill Livingstone (pub) Edinburg, London and New York. Vol II.

Dilika, NF. ; NI Kolova, RV.; Jacobs, TV. (1996).Plants used in the circumcision rise of the Xhosa tribe in South Africa. International Sympsium on Medicinal and Aromatic Plants.27 (30) 165-169. S

Douglas, J.; Mackay,A; Miller. L. (2003). Nutritional support for wound healing. Alternative Medicine Review. 28(10):1-3

Elghazali, GEB. ; EA.; Bashir, AK; Salih, AKM. (1987). Medicinal Plants of the Sudan: Medicinal plants of the Eastern Nuba Mountains. Part II. Khartoum, Khartoum University Press. Sudan

Elghazali, GEB. ; Eltohami, MS.; Elegami, AAB. (1994): Medicinal plants of the Sudan: Medicinal plants of the White Nile Provinces. Part III. Khartoum, Khartoum University Press.Sudan.

Elghazali, GEB. (1997).Sudanese Promissing Medicinal Plants.Omdurman Islamic University Press (Pub.)Sudan

El-Hadi, MMM.; Hassan, SK.; Yousif, GM. (2005): Antimicrobial activity of *Nigella sativa* L.seeds oil. Fitoterapia, 1(1): 103-116.

Elmer, WK.; Steven, DA.; Dwell, VR.; Willam,MJ.; Herbert, MS.; Washington, CW (1990).Colour Atlas and Text book of Dignostic Microbiology. Third edition, Lippincott Company, Philadelphia, 473-475

Faleiro, ML. ; Miguel, MG.; Ladeiro, F.; Venancio, F.; Tavares, R.; Brito, JC.; Figueiredo, AC.(2003). Antimicrobial activity of essential oils isolated from Portuguese endemic species of Thymus. Lett Appl Micobiol, 36(1): 35-4

Garg, S.; Chhabra, R.; Talwar, GB.(1994). Neem from ethnomedicine to modern system of medicine. Ethnobiology in Human welfare: abstracts of the fourth international congress of ethnobiology, Lucknow, India, 17-21.

Grace, OM.; Light, ME.; Lindsey,KL.;Standen,(2002). Antimicrobial activity and isolation of active compounds from fruit of *Kigelia africana***.**South Africa Journal of Botany.68 (2):220-222.

Grand, AL. (1989).Anti-infectious phytotherapies of the tree-savanna, Senegal (West Africa).Journal of Ethnopharmacology. 25(3):315-338.

Hamill, FA; Apio, NK. ; Bukenya, Z R.; Monsango, M.; Maganyi, OW; Soejarto, DD. (2003). Traditional herbal drugs of Southern Uganda, literature analysis and antimicrobial assays. Journal of Ethnopharmacology, 84(1): 57-78.

Haraguchi, H. (1998*)*. Mode of action of antimicrobial substances in plants. Recent Research Developments in Agricultural and Food Chemistry, 2(1): 259-268.

Hardman, JG.; Limird, LE.; Molinoff, PB.; Puddon, RW.; Gilman, AG.(1996).The Pharmacological Basis of Therapeutics. 9th Edition. McGraw Hill. New York

Harries, M. (1964). Pharmaceutical Microbiology. Mailiene, Tindal and Cox. London.

Hawkey, PM.; Lewwis, DA. (1989). Medical Bacteriology, a practical approach, first edition, Oxford University Press, Oxford.

Ibrahim, LF. ; Kawashty, SA.; El-Eraky, WI.; Shabana, MM.; El-Negoumy, SI.(2000). Acomprative study of the flavonoids, pharmacological and antimicrobial effects. Egyptian Journal of Physiological-Sciences, 24(1): 63-82.

Ijah, UJJ and Oyebanji, FO. (2003). Effect of Tannins and Polyphenols of some Medicinal plants on bacterial agents of urinary tract infection. Global journal of Pure and Applied Sciences, 9(2): 193-198.

Jovel, EM .; Cabanillus, j.; Towers, GHN. (1996) .An ethnobotanical study of the traditional medicine of the Mestizo people of Suni-Marino. Journal of Ethnopharmacology, 53(3): 149-156.

Kakali., S.; Mukherjee, PK.; Das,J.; Pal,M.(1997).Wound healing activity of *Leucas eavadulaefolia* Rees. Journal of Ethnopharmacology. 56(2): 139-144.

Kavanagh, F. (1972). Analytical Microbiology. Vol II, Academic Press (Pub), New York and London.p.11

Kay, MA. (1996). Healing with plants in the American and Mexican West, First edition, Arizona, University of Arizona press.

Khan, MT.; Ashraf, M.; Bukhtair, MK.; Ashraf, S. (1993) Antimicrobial activity of *Withania coaulans*. Fitoterapia 4 (1) 431-436.

Kostova, I. (2001). *Fvaxinus ornusl*. Fitoterapia. 72(5): 4

Kwo,Vt.;Craker,LE.;Nolan,L.;Shelly,K.(1996). Screening Camerron MedicinalPlant extracts for antimicrobial activity.Acta Horiculturare.42 (6):147-155.

Mahasneh, AM.; Abaas, J A .; El-oqlah, AA.(1996). Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Bahrain. Phytotherapy Research, 10(3):251-253.

Manhal, H; Almagboul, AZ.; Omer, MEA. ; Elshiekh, OM. (2004). *In vitro* antibacterial activity of *Pistacia lentiscus* against some human bacterial infections. Journal of Science and Technology, 5(1):65-72.

Martino, V.; Caffini, N.; lappa, A.; Ferraro, G.; Schilcher, H.; Phillipson, JD. (1999). Second world congress on medicinal and aromatic plants for human welfare. Acta-Horticulturae, 501:336.

Martins, AP.; Salgueiro, LR. ; Vila, R.; Gunha, A.(2002) .” Essential oil composition and antimicrobial actitivity. Planta Medica. 69(1) 77-79.

Mathieu, D.; Watell. F.; Billard, V.; Defoin, IF. (1999). Post traumatic limb ischemia. Journal of Trauma.30 (3): 307-314.

Merzouki, A.; Derfoufi, F.; El-Aallali, A.; Molero, MJ. (1997). Wild medicinal plants used by local Bouhmed population (Morocco). Fitoterapia, 68(5): 444-460.

Michel,DA.and Demontaig,NE.(1993).Medicinal plants. Fitoterapia. Volume 54(1): 5-8.

Miles, M. and Misra, SS. (1938). The estimation of the bactericidal power of the blood. Journal Hyg. 8(3): 732.

Mohammed, TO. ; Almagboul, AZ.; Omer, MAA. ; Abd Rabo, AN.; Ahmed, SOM. (2006). *In vitro* antimicrobial activity of *Borreria sinesis.* Omdurman Journal of Pharmceutical Sciences 1(2): 188-199.

Nagappa, AN.; Cheriyan, B. (2002). Wound healing activity of the aqueous extract of *Thespesia populnea* fruit. Fitoterapia. 72(5): 503-506.

Nayar, Sr.; Balagopian, TP; Raveevdran, NS. (1994).Clinical traial with Himax lotion on wounds in large animals.Indian Journal of Indigenous Medicines. 11(1): 61-62.

Nexon, W. (1951). Polyethylene Glycol. Parma. J. 16(7): 213.

Nwosu, MO. (1999).The use of herbal remedies against Psychiatric ailments in Tropical West Africa. Fitoterapia, 70(1):58-63.

Palanichamy, S.; Bhaskar, Ea.; Bakthava thsal, R. (1992). Wound healing activity of *Cassia alate*.Fitoterapia. 62(2): 153-156.

Patric, RM.; Ken, SR.; Michael, AP. (2005). Medical Microbiology, Fifth edition, Library of Congress, Philadelphia, 203-212

Pattel, NK and Foss, NE. (1964): Polyethelene Glycol.Journal Pharm. Sci. (3): 367-369.

Pieters, L.; Bruyne, T.; Poel, BV. ; Vingerhoets, R.; Totte, J.; Berghe, D. (1995). *In vivo* wound healing activity of Dragon's blood(croton spp.), A traditional South American drug, and some of its constituents. Phytomedicine, 2(1): 17-22.

Phillipson, JD. (1997). Biology in action: Medicinal plants. Journal of Biological Education, 31(2): 109-115.

Poole, TB and Robinson, R. (1989): The Hand bookon the care and mangement of laboratory animals. 6th Edition. Longman Scientific and Technical (Pub). London.

Omer, MEA. (2000). Medicinal and Aromatic Plants as a Source of Herbal Medicines, National Symposium on the Research Application in the iIndustrial Sector(ISESCO).16-18 October, Khartoum, Sudan.

Owolabi, OJ andOmogabi, EK.(2007). Ethanolic stem- bark extract of *Kigelia africana*. African Journal of Biotechnology. 6(5) 582-585.

Ramesh, N.; Viswanathan, MB.; Saraswathy, A.; Balakrishna, K.; Brindha, P.; Lakshmanaperumalsamy, P.(2001). Phytochemical and antimicrobial studies of *Bridelia crenulata*. Pharmaceutical Biology, 39(6): 460-464.

Rao, SG. ; Udupa, AL.; Udupa, SL.; Kulkarni, DR. (1991): Calendula and Hypericum: Two homeopathic drugs promoting wound healing in rats.Fitoterapia, 62(6): 508-510.

Rasik, Am.; Ram, R.; Gupta, A.; Shukla, A.; Dubey, MP.; Srivastava, S.; Jain, HK.; Kulshrestha,DK.; Raghubir, R.(1999) Healing potential of *Calotropis procera*. Journal of Ethnopharmacology, 1(3): 261-266.

Robinson, J.; Gibaldi, M.; Weiner, ND. ; Kanig , J I. ;(1964). Polyethylene Glycol, Journal of Pharm. Sci .5 (3): 1245-1247.

Rode, H.; Wet, PM.; Cywes, S. (1989).The antimicrobial effect of *Allium sativum* L. (garlic). South African Journal of Science, 85(7): 462-464.

Rukangira and Ernest. (2001). The African herbal industry constrains and challenges.The natural products and cosmeceuticals conference. Erboristeria Domani (Pub.).

Salle, AT. (1961). Fundamental Principal of Bacteriology. 5th Edition McGraw hill Book CO (Pub.). London.

Sanogo,R.;GrisafiG.;Germano,Mp.;Depasquale,R.;Bisignano,G.;Cappaso ,F;.Basso,F;Evanos,FJ.;Mascolo,N.(1998).Evalulation of Malian traditional medicines.Phytotherapy research.12(1):5154-5156.

Shayoub, MAA. (1985). Polyethylene Glycol ointment bases evaluations and application in Sudan, Master Thesis, and University of Khartoum, Sudan.

Shukla, A.; Rasik, AM.; Shankkar, R.; Dhawan, BN. (1999). *Invitro* and *in vivo* wound healing activity of asiaticoside isolated from *Centella asiatica*. Journal of Ethnopharmacology. 65(1): 1-11.

Sidhu,GS.; Singh, AK.; Banaudha, KK.; Gaddipata, JP.; Patnaik, GK.; Maheshwari,PK. (1999). Arnebin-1 accelerates normal wound healing. Journal of Investigative Dermatology, 113(5): 773-781.

Silva, O and Gomes, ET. (2003). Guieranone A naphthyl butanone from the leaves of *Guiera senegalensis* with antifungal activity.Journal of Natural Products.66 (3):447-449.

Thang, PT.; Patrrick, S.; Teic, LS. ; Yung, CS.(2001). Anti-oxidant effect of the extracts from the leaves of *Chromolaena odorata* on human dermal fibroblasts. Burns, 27(4): 319-327.

Tego, G.; Stermitz, FR.; Lomovskaya, O.; Lewis, K. (2002). Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agents and Chemotherapy, 46(10): 3133-3141.

Thom, SR.; Bolotin, T.; Hardy, K.; Nebolon, M.; Kilpatric, L. (1997).Inhibition of human neutrophil. Amercan Journal of physiology. 3(1):770-777.

Ueng, TH; Kang, JJ; Wang, HW; Lin, PC. (1997): An overview of the toxicology of commonly used traditional Chinese medicine. Journal of Food and Drug Analysis 5(4): 241-264.

Valsaraj, R.; Pushpangadan, P; Smitt, UW; Adsersen, A. (1997). Antimicrobial screening of selected medicinal plants from India. Journal of Ethnopharmacology, 58(2):75-83

Viller, AM; Schorn, PJ; Abatangelo, G; Nencini, P; Baradli, M. (1998). IX Congress of the Italian Society of Pharmacognosy. Fitoterapia, 5: 82.

Warren, MD and Ernest, MD. (2002). Medical Microbiology and Immunology, Seventh edition, Donnelley and Sons Company, New York 59-80.

Yuh, FC; Huei, YT; Tian, SW. (1995). Antimicrobial and analgesic activities from roots of *Angelica pubescens.* Planta Medica: 2(8), 2-8.