بسم الله الرحمن الرحيم

ANTIMICROBIAL AND WOUND HEALING ACTIVITY

OF SOME SUDANESE MEDICINAL PLANTS

A THESIS SUBMITTED BY
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DEDICATION

TO MY MOTHER, HEAVENS OF LOVE

TO MY FATHER, LAND OF PATIENCE

TO MY SISTER, LAKE OF JOY

TO MY BROTHER, WIND OF EAGERNESS

TO MY TEACHERS, RIVERS OF GIVING

TO MY FRIENDS, FLOWERS OF HAPPINESS

TO MY FIANCEE, FRAGRANCE OF HOPE

ACKNOWLEDGEMENTS

ABSTRACT

A total of 30 plant extracts belonging to 10 Sudanese medicinal plants, distributed among 10 families were screened for their antimicrobial activity using the cup plate agar diffusion method. They were tested against five standard bacteria, two Gram positive bacteria (*Bacillus subtilis* NCTC 8236 and *Staphylococcus aureus* ATCC 25923) and three Gram negative bacteria *(Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380 and *Pseudomonas aeruginosa* ATCC 27853).*

26 extracts (86.6%) exhibited inhibitory activity against one or more of the five tested bacteria. Out of the 26 extracts showing antibacterial inhibitory effects, 8 (30.8%) were inhibitory to the 5 tested organisms.

Staphylococcus aureus was the most susceptible organism to the extracts, while *Proteus vulgaris* showed the least susceptibility.

The minimum inhibitory concentrations (MICs) of the most active methanolic extracts of the 10 plants against the standard bacteria were determined using the agar plate dilution method.

The antibacterial activity of four reference drugs were determined against the five tested Gram positive and Gram negative bacteria, and compared to the antibacterial activity of the tested plant extracts.

The most active extracts against the standard bacteria were selected, they were (18); belonging to the 10 plants; then they were tested against 100 clinical isolates collected randomly from the National Health Laboratory, Khartoum Teaching Hospital, Police Teaching Hospital and Khartoum-North Teaching Hospital.

The antifungal activity of the 30 extracts was investigated against two fungi (*Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596). Out of the 30 extracts screened, 16 (53.33%) exhibited inhibitory activity against one of the two tested fungi. 6 extracts (20%) exhibited inhibitory effect against the two tested organisms. *Candida albicans* was the most sensitive organism, while *Aspergillus niger* showed the least susceptibility.

The minimum inhibitory concentrations (MICs) of the most active methanolic extracts of the 10 plants against the standard fungi were determined using the agar plate dilution method.

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The antifungal activity of two reference drugs were determined against the two tested fungi, and compared to the antifungal activity of the tested plant extracts.

Solenostemma argel is one of the most widely used medicinal plants in Sudan, and is employed in numerous traditional preparations. In this study the wound healing effect of *Solenostemma argel* leaves extract was investigated on open skin wound model on rats. 30 Swiss Wistar Albino rats of either sex weighing 80-100g 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 ointments were applied twice daily.

Two trials were performed; the first using three groups of non-infected rats and the second using three groups of artificially infected rats with standard *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 Tetracycline ointment 3%.

ملخص الاطروحة

أشتملت الدراسه علي أجراء مسح للفاعليه المضادة للبكتريا ل 30 خلاصة نباتية من 10 نباتات طبية سودانية ، تنتمي الي 10 عوائل بطريقة الانتشار في الأجار. تم أختبار تأثير الخلاصات ضد خمسة أنواع من البكتريا المعياريه، نوعين من البكتريا الموجبة الجرام (العصوية الرقيقة و العنقودية الذهبية) وثلاثة أنواع من البكتريا سالبة الجرام (الاشريكية القولونيه، الزائفة الزنجبارية و المتقلبة الاعتيادية).

وجد أن 26 خلاصة (86.6%) أظهرت فاعلية ضد واحد أو أكثر من أنواع البكتريا المختبرة، كما أتضح أن 8 (30.8%) من ال 26 خلاصة لها مفعول مثبط لآنواع البكتريا الخمسة. كانت العنقودية الذهبية أكثر أنواع البكتريا حساسية للخلاصات، أما المتقلبة الاعتيادية فقد أظهرت أقل حساسية بين أنواع البكتريا المختبرة.

أشتملت الدراسة علي تحديد أقل تركيز مثبط لنمو البكتريا المعيارية لاكثر الخلاصات فاعلية، وهي ال10 خلاصات الميثانولية، بطريقة تخفيف الاجار.

تم تحديد فاعلية أربعة مضادات حيوية مرجعيه ضد الخمسة بكتريا المعياريه، وقورنت فاعليتها مع فاعلية خلاصات النباتات المختبرة ضد البكتريا المعيارية.

أختيرت أكثر الخلاصات فاعلية ضد البكتريا المعياريه وهي (18) خلاصة نباتية من ال 10 نباتات الطبية السودانية، ومن ثم أختبرت هذه الخلاصات ضد 100 عينة بكتيرية معزولة من مرضي. تم عزلها عشوائيا بالمعمل القومي الصحي، مستشفي الخرطوم التعليمي، مستشفي الشرطة التعليمي و مستشفي الخرطوم بحري التعليمي.

تم البحث في الفاعلية المضادة للفطريات ل 30 خلاصة ضد أثنين من الفطريات المعياريه (المبيضة البيضاء و الرشاشية السوداء).

وجد أن 16 خلاصة (53.33%) من ال30 خلاصة التي تم أجراء المسح لها أظهرت مفعول مثبط ضد واحد من نوعي من الفطريات المستخدمة. كما أن 6 (20%) خلاصة منها أظهرت مفعول مثبط ضد الفطرين المختبرين.

المبيضة البيضاء كانت أكثر الكائنات حساسية، بينما الرشاشية السوداء كانت أقلها حساسية.

أشتملت الدراسة علي تحديد أقل تركيز مثبط لنمو الفطريات المعيارية لاكثر الخلاصات فاعلية، وهي ال10 خلاصات الميثانولية، بطريقة تخفيف الاجار.

تم تحديد فاعلية مضادين حيويين مرجعيين ضد الفطرين المعياريين، وقورنت فاعليتها مع فاعلية خلاصات النباتات المختبرة ضد الفطرين المعياريين.

يعتبر نبات الحرجل أحد النباتات الطبية واسعة الاستخدام في السودان ويستخدم في العديد من المستحضرات التقليدية. وقد تم في هذه الدراسه التحقق من تأثير الخلاصة الميثانوليه لنبات

الحرجـل علي ألتئـام جـروح الجلـد المفتوحـة فـي 30 مـن الفئـران البيـضاء السويـسرية، حيـث أستخدمت الفئران من كلا الجنسين وباوزان تتراوح بين 80-100 جرام، بعد حلق شعر مؤخرة الظهـر والجانـب الايمـن وأحداث جرح غـائر دائـري قطره 1 سـم فـي المنطقـه المحلوقـه. وتـم تحضير خلاصه ميثانولية من أوراق النبات. كمـا تم أيضا تحضير مرهم 2% (وزن/وزن) من الخلاصـة فـي البـولي أيثـايلين جليكـول. مـع أستخدام مـرهم التتراسيكلين 3% كحكـم، كـل مـن المرهمين تم مسحه مرتين يوميا.

تـم عمـل تجربتين، الاولـي مكونـة مـن ثلاثـة مجموعـات مـن الفئـران غيـر المـصابه والاخـري مكونـة من ثلاثة مجموعـات من الفئران المصابه معمليا بالعنقودية الذهبية المعيارية.

قورنت المجموعات المعالجة مع المجموعات غير المعالجة حيث تم تقدير الالتئـام بالنقص فـي منطقة الجرح. وأكدت النتائج أن مرهم أوراق الحرجل 2% هو عامـل التئـام فعـال، بل وجد أنـه أفضل من مرهم التتراسيكلين 3% المختبر.

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INTRODUCTION
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CHAPTER ONE
INTRODUCTION

1.1 INTRODUCTION

Since the dawn of history, Mankind started to recognize surroundings, and tried to discover the secrets of nature.

Mankind found plants of unlimited uses: Food, beverages, shelter, cloth, ornaments and tools for protection etc…

Disease was, and still, enemy number one against human beings and their animals, so it was of sense for the first Man to focus on plants as a reliable, safe, effective and available source of medication.

The use of plants as remedies dates back to the ancient Pharonic Egyptians Era (3000 B.C.), whereas evidences of plants were seen inside the graveyards with the mummified bodies.

In China (2700 B.C.), appeared the first Medicinal plants book, which was the bases for the following books like **' The Great Herbal'.**

In the Romanic Era, **Materia Medica** appeared as a reference book, which included 958 plants with description of their uses (Saad *et al*, 1988).

The importance of Traditional Medicine as a source of Primary Health Care (PHC) was first officially recognized by the World Health Organization (WHO) in the primary health care declaration of Alma Ata (1978) and has been globally addressed since (1976) by the Traditional Medicine Programme of the WHO (Rukangira, 2001).

Traditional medicine, in one form or another, is widespread throughout the world. As its name implies, it is part of the tradition of each country, where it is handed down from generation to generation. Its acceptance by a population is largely conditioned by cultural factors and much of traditional medicine, therefore may not be readily transferred from one culture to another (Akerele, 1991).

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 to generation, whether verbally or in writing" (Rukangira, 2001).

Herbal Medicine is also defined as: "A plant derived material or preparation with therapeutic or other human health benefits which contains either raw or processed ingredients from one or more plants. In some traditions, materials of inorganic or animal origin may also be present" (WHO, 1993).

WHO has described Traditional Medicine as "One of the surest means to achieve total health care coverage of the world's population" (Rukangira, 2001).

WHO estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also modern pharmacopoeias still contain at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Schmincke, 1997).

World Health Organization prepared a list of 20.000 medicinal plants used world wide, and indicated that 4.000 drugs from plant origin are used in a wide range worldwide (Table No.1). In West Europe only, there are about 400 medicinal plants marketed widely (Omer, 2000b).

What is significant is the growing recognition from the industrialized world, that these so-called *Traditional Values* are valid for all people, responding to the environmental and ecological deterioration that threatens health and development everywhere (Akerele, 1991).

One must also keep in mind that many people in Europe, the UK and USA are turning to alternative medicine to some extent, because of the side effects induced by powerful, synthetic allopathic drugs. Herbal medicine is one of the alternatives people are turning to (Chaudhury, 1992).

Nearly half of all prescription drugs produced in Germany are initially derived from raw plant materials; and in USA, over 1/4 of the 1.500 million prescriptions dispensed annually, are derived from plants (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 traditional

(TableNo.1)

SOME IMPORTANT PLANT-BASED INGREDIENTS OF MEDICAMENTS

Ingredient	Plant species	Application		Climate zone	Main product basis
		Industrial	Traditional		
Aspirin	*Salix alba*	Pain-killer		Temperate	Synthetic
Atropine	*Atropa belladonna, Duboisia myoporoides*	Pupil dilatation, bradycardia, Parkinson's disease, asthma, traveller's diarrhoea	Same	Tropical	Synthetic
Digoxin	*Digitalis sp.*	Heart failure, arrhythmia	Same	Temperate	Synthetic
Ephedrine	*Ephedra sinica*	Nasal decongestant	Same	Temperate	Synthetic
L-Dopa	*Mucuna deeringiana*	Treatment of Parkinson's disease	Same	Tropical	
Picrotoxin	*Anamirta cocculus*	Nervous system stimulus	Same	Tropical	Plant source
Pilocarpine	*Pilocarpus jaborandi*	Treatment of glaucoma	Same	Tropical	Plant source
Quininde	*Cinchona sp.*	Treatment of arrhythmia	Same	Tropical	Plant source
Quinine	*Cinchona sp.*	Antimalarial	Same	Tropical	Synthetic
Reserpine	*Rauwolfia serpentina*	Antihypertensive agent	Same	Tropical	
Scopolamine	*Hyoscyamus niger*	Treatment of motion sickness	Same	Tropical	Plant source
Taxol	*Taxus brevilifolia*	Ovarian cancer		Temperate	Plant source
Vinblastine	*Catharanthus roseus*	Treatment of Hodgkin's disease		Tropical	Plant source
Vincristine	*Catharanthus roseus*	Childhood leukaemia		Tropical	Plant source

(Source: Rukangira, 2001)

Practitioner there are 224 individuals, compared to one trained doctor for nearly 21.000 individuals (Rukangira, 2001).

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 mostly to satisfactory results (Boulos, 1983).

The attention paid by health authorities and administrations to the use of medicinal plants has increased considerably (Akerele, 1991).

The proper use of medicinal plants is a necessity, not a luxury (Akerele, 1991).

The main problem facing the use of traditional medicines: is the proof requirement that the active components contained in medicinal plants are useful, safe and effective (Rukangira, 2001).

One of the main obstacles to fuller cooperation between traditional and western systems is the residual suspicion among many western professionals that charlatanism is endemic in the traditional context. The fact that traditional medical techniques and remedies have frequently not lent themselves to quantification, and have not yet been tested, has reinforced this fear. But in the more realistic climate that is now developing, steps are increasingly being taken to examine the merits of the traditional medicine in the light of modern sciences, with a view to adopting effective medical practices and discouraging the harmful ones (Ayensu, 1978).

The contrast between the situation in developing countries and that of the developed world presents a challenge to the national health authorities whereas it is acknowledged that the tropics are a rich source of plants with medicinal properties, the study and knowledge of their properties still remain largely in the hands of the industrialized countries (Akerele, 1991).

A major factor impeding the development of the medicinal plant based industries in developing countries has been the lack of information on the social and economic benefits that could be desired from the industrial utilization of medicinal plants (De Silva, 1997).

Africa has a long and impressive list of medicinal plants. For example: *Securidaca longepedunculata* is a tropical plant found almost everywhere in Africa. The dried bark and root are used in Tanzania for nervous disorders. One cup of its root decoction is administered daily for two

weeks. Throughout East Africa; the plant's dried leaves are used for wounds, coughs, venereal diseases and snake bites. In Malawi, the leaves are used for wounds and headaches, while in Nigeria they are used for skin diseases (Rukangira, 2001).

This plant is also found in Sudan, and will be tackled in this study.

Documentation of Medicinal uses of African plants is becoming increasingly urgent because of the rapid loss of the natural habitat for some of these plants due to anthropogenic activities. Many of the Medicinal plants and other materials become extinct before they are even documented (Rukangira, 2001).

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 concoctions 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 Alkaloids in plant families such as the *Nightshade*, the *Poppy* and the *Pea* have been well known for healing qualities (if not in name, then in substance) 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).

Skin care in Africa is undertaken under several different practices. Among the common practices is the skin care for beauty is additional to care against wounds (Rukangira, 2001).

In Sudan the situation is very similar to that in the African countries.

The overall effect of any scientific study will help to variously establish and confirm the credibility of the use of herb and medicinal plants as an effective source of both traditional and modern medicine, and this what has been attempted to explore in this study.

1.1.1. AIMS OF THIS WORK

Objectives of the work are as follows:

1- To enrich the information about the antimicrobial activity of Sudanese medicinal plants.

2- To verify the claimed activity of certain Sudanese medicinal plants used in the traditional medicine as antimicrobial agents.

3- To subject these plants for further antimicrobial studies i.e. to test them against clinical isolates, to determine the minimum inhibitory concentration (MIC) and to compare them with the commonly used antimicrobial agents in the Sudan.

4- Establishment of *in vivo* Antimicrobial Activity Assessment Model (for the Medicinal Plants), using Rats, throughout evaluation of the wound healing activity of one of the potent plants under study.

1.2 LITERATURE REVIEW

1.2.1. Antimicrobial activity of Medicinal plants:

The use of Medicinal plants as anti-infective agent is very well documented.

Nicollas *et al.* (1973) showed that a wide range of organisms including *Aspergillus fumigatus* and *Candida albicans*, were susceptible to plant extract containing passicol in a qualitative paper disk test. Passicol was non toxic to mice and rabbits when administered by intravenous injection.

Di Salvo (1974) reported that an extract of *Baccharis glutinosa* leaves was tested against *Candida albicans, Cryptococcus neoformans* and *Histoplasma capsulatum*. All of the microorganisms were resistant to the extract.

Prokopchuk *et al.* (1979) screened extracts of 29 plants, which showed marked fungistatic action.

Ross *et al*. (1980) selected the alcoholic extracts of 32 plants on the basis of literature references and use in folklore, and studied them for their activity against four pathogenic bacteria and five fungi. Only eight of the plants, including *Peganum harmala* and *Tamarindus indica*, showed much antibacterial activity. Whereas five, including *P. Harmala* and *Solenostemma argel,* were markedly antifungal. There was a correlation between the antimicrobial activity and the contents of physiologically active principles.

Abdel Nasser *et al.* (1983) studied the effect of dry residues from plants of different families on bacteria *in vitro* and *in vivo*. Dry residues of Paprica leaves, Tomato tops, Egg plant leaves, Guava leaves, onion peels, Garlic tops, Wheat straw, Sugar cane leaves, Cotton leaves, Egyptian clover tops, Field bean tops or pea tops were examined for the presence of antibacterial substances, using successive extractions with hexane, ethyl ether, ethanol and water respectively. On culture media the antibacterial effect, expressed as width of inhibition zones, differed according to the type of plant, type of micro-organism and extraction medium. Water extract from the plants showed no effect on any of the microorganisms studied. But of the other extracts, ether extract showed the highest antimicrobial activity. The antibacterial substances seemed to be more inhibitory to Gram (+) bacteria.

Farouk *et al.* (1983) screened 76 extracts of 31 Sudanese plants belonging to 21 families for their antibacterial activity against four bacteria. Out of the 76 extracts, 64 exhibited inhibitory effects against at least one microorganism. Of these, seven plants showed significant inhibition of tested microorganisms.

Almagboul *et al.* (1985a) investigated 198 extracts of 40 Sudanese plants belonging to 18 families for their antibacterial activity against four different bacterial species. Out of 198 extracts, 134 (68%) exhibited inhibitory effects against one or more microorganisms.

Almagboul *et al.* (1985b) studied 135 extracts of 31 Sudanese plants belonging to 15 families for their antibacterial activity against four different bacterial species. Out of 135 extracts, 101 (75%) exhibited inhibitory effects against one or more microorganisms. The plant extracts which showed marked antibacterial effect were phytochemically screened.

Almagboul *et al.* (1985c) tested 126 extracts of 24 Sudanese plants belonging to 21 families for their antibacterial activity against four different bacterial species. Out of 126 extracts, 97 (77%) exhibited inhibitory effects against one or more microorganisms. The plants which showed marked antibacterial activity were shown to be rich in flavanoids, tannins and alkaloids.

Saxena and Vyas (1986) screened the petroleum ether and ethanol extracts of seeds of 14 Indian plants for their *in vitro* activity against human pathogenic fungi (*Aspergillus fumigatus, Trichophyton mentagrophytes* and *Candida albicans*) and bacteria (*Escherichia coli, Bacillus subtilis* and *Streptococcus faecalis*). Antifungal activity was demonstrated by four plants including *Raphanus sativus*. The four plants also demonstrated antibacterial activity.

Tharib *et al.* (1986) screened four compounds isolated from the stems of the desert shrub *Solenostemma argel*, which is used in local medicine in Libya. Only one (from the saponifiable fraction) showed activity against both Gram positive and Gram negative bacteria.

Mori *et al.* (1987) tested (-)- Epigallocatechin, which was isolated from the Japanese medicinal plant *Elaeagnus glabra,* along with other 28 related plant flavonoids, for their antibacterial activity.
(-)- Epigallocatechin was active against *Staphylococcus aureus* and *Protus vulgaris.*

Thakur *et al.* (1987) reported that the ether and chloroform extracts of three out of six tested Indian plants exhibited satisfactory antifungal activity against three dermatophytes.

Almagboul *et al.* (1988a) investigated 102 extracts of 15 Sudanese plants belonging to six families for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*. Out of 102 extracts, 87 (85%) exhibited inhibitory effects against one or more microorganisms.

Almagboul *et al.* (1988b) tested 102 extracts of 18 Sudanese plants belonging to 12 families for their antifungal activity towards *Aspergillus niger* and *Candida albicans*. Out of 102 extracts, 32 (31.37%) exhibited inhibitory effect against the two mentioned fungi.

Biyiti *et al.* (1988) found that the decoctions of stem bark of the Cameroonian plant *Erythrina sigmoidea*, which is used to treat Gonorrhoea and Vagainitis, showed antibacterial activity towards all tested Gram positive bacteria, but it was inactive against the Gram negative and Fungi tested.

Grand (1989) reported the result of a literature survey of the ethnobotanical and pharmacological data concerning 43 species used by the Diola against Fungal and Bacterial infections. 10 of these plants, including *Guiera senegalensis*, showed antimicrobial activity which he associated with the presence of pharmacologically active substances.

Almagboul (1992) reported *Solenostemma argel* as one of the plants which exhibited antimicrobial activity in her study.

Almagboul (1992) screened the antibacterial activity of some Sudanese medicinal plants including: *Kigelia africana, Xeromphis nilotica* and *Securidaca longepedunculata*. She tested the chloroformic, methanolic and aqueous extracts of each of these plants against *Bacillus subtilis, Staphylococcus aurues, Escherichia coli* and *Pseudomonas aeruginosa.* The fruit chloroformic and methanolic extracts of *Kigelia africana* gave good results, while the aqueous extracts were inactive against the tested organisms. The fruit chloroformic and methanolic extracts of *Xeromphis nilotica* were only active against *S. aureus*, but the fruit aqueous extract was active against all tested organisms. All leaves extracts of *Securidaca longepedunculata* were active against the tested organisms.

Heisey and Gorham (1992) tested the extracts of 54 plant species for ability to inhibit bacteria and fungi (mainly *C. albicans, T. rubum* and *S. mutans*). A total of 15 plant extracts produced detectable antimicrobial activity.

Avila *et al.* (1993) evaluated the antimicrobial activity of six medicinal plants used by Purepecha in (a Mexican ethnic group) for the treatment of gastrointestinal diseases. Three of these plants showed antimicrobial activity against *Escherichia coli* ATCC25922 and *Salmonella typi* ATCC19430.

Udda *et al.* (1993) concluded that the antimicrobial properties of water soluble products obtained by degradation of 4-methylthio-3-butenyl isothiocyanate, the pungent principle from radish (*Raphanus sativus*) were studied using five Gram negative and five Gram positive bacteria, five yeasts and nine fungi. Two antimicrobial components were found in the water soluble products. The identified compounds exhibited a prominent growth inhibition on the fungi and Gram positive bacteria.

Elhady *et al.* (1994a) studied the antimicrobial activity of the aerial parts of *Solenstemma argel*, which were successively extracted with methanol/water in different proportions (four fractions). Eight bacteria were used: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Micrococcus* and *Streptococcus spp.* 14 fungi were tested including: *Aspergillus niger* and *Candida albicans.* The greatest effect was observed by *Streptococcus* species and moderate action against *E. coli, S. aureus, K. pneumoniae* and *P. vulgaris.* Fraction No. one showed antifungal activity against *A. niger,* while fraction No. two showed activity against *C. albicans.* Fration No. three showed activity against both fungi, while fraction No. four showed high activity against *A. parasiticus* and *A. candidus.*

Elhady *et al.* (1994b) studied the antimicrobial activity of the aerial parts of *Solenstemma argel*, which were successively extracted with chloroform/methanol in different proportions (four fractions). Eight bacteria were used: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Micrococcus* and *Streptococcus spp.* 14 fungi were tested including: *Aspergillus niger* and *Candida albicans.* The greatest effect was observed by *Streptococcus* species and moderate action against *E. coli, S. aureus, K. pneumoniae* and *P. vulgaris.* There was no effect on Micrococcus and *Pseudomonas aeruginosa,* while a weak fungicidal activity was observed.

Alkofahi *et al.* (1996) examined the ethanolic extracts of 52 Jordanian medicinal plants for their antimicrobial activity. 26 showed antibacterial and antifungal activity against one or more of the six tested microorganisms.

Ela *et al.* (1996) evaluated the antimicrobial activity of 16 essential oils, from Egyptian plants including *Raphanus sativus*, against *Escherichia coli, Staphylococcus aureus*, *Aspergillus niger, Candida albicans* and *Chaetomium olivacum.* All the essential oils studied showed potent activities against at least one of the tested organisms.

Kwo *et al.* (1996) screened the concentrated bark extracts of three Cameroonian medicinal plants (*Alstonia boonei, Kigelia africana* and *Morinda lucida*), for their antimicrobial activity using the disk diffusion susceptibility test. Solvents with different polarity were used for the extraction (methylene chloride, ethyl acetate, 95% ethanol and acetonitrile), and the extracts were tested against five human pathogens: *Candida albicans, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis (Streptococcus faecalis)* and *Pseudomonas aeruginosa.* The patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. The largest zones of inhibition were observed for ethanol extracts of *kigelia africana* against *Staphylococcus aureus and Pseudomonas aeruginosa. Staphylococcus aureus* was the most inhibited microorganism. No inhibitory effects were observed against *C. albicans*. The extent of inhibition of the bacteria was related to the concentration of the plant extract.

Wolski *et al.* (1996) tested the antifungal activity of *Archangelica officinalis* (a medicinal plant from Poland) extract against nine fungal species and compared the results with the activities of Amphotericin B and Clotrimazole; data were tabulated. The antifungal activity was directed towards dermatophytes (MIC 6.25-12.5 μg/ml).

Almgboul *et al.* (1997) isolated two new compounds from *Vernonia amygdalina* Del. (Asteraceae): Vernolepin and Vernodalin, and tested their antimicrobial activity against four bacteria and two fungi using the disk diffusion method. The two isolated compounds were highly active against the tested bacteria and fungi.

Darokar *et al.* (1997) detected the antimicrobial activity in the floral petals of 20 plant species belonging to 12 families from India.

Gao *et al.* (1997) performed primary studies on the bacteriostasis and fungistasis of lysozyme from *Raphanus sativus*. Growth was inhibited by varying degrees.

Omer *et al.* (1997) investigated 135 extracts of 32 Sudanese plants belonging to 19 families for their antibacterial activity against four different bacterial species. Out of 135 extracts, 114 (85%) showed significant antibacterial activity, thus supporting the use of the plants in traditional medicine.

Silva *et al.* (1997) studied the *in vitro* antibacterial activity of eight medicinal plants from Guinea-bissau including: *Guiera senegalensis*, against *Neisseria gonorrhoeae*. These plants are used by Fulani traditional healers to treat several disorders including venereal diseases. All extracts exhibited antibacterial activity against all the tested strains of *Neisseria gonorrhoeae*.

Zhang and Lewis (1997) isolated two new antimicrobial peptides by acid extraction from the broad bean *Vicia faba*. The extract was separated by ion exchange chromatography, and a fraction showing antibacterial activity was further purified by reverse-phase HPLC. The new peptides were named fabatins. Fabatins were active against both Gram negative and Gram positive bacteria, but were inactive against the yeasts *Saccharomyces cerevisiae* and *Candida albicans*.

El-egami *et al.* (1998) tested 114 extracts of 35 Sudanese plants for their antibacterial activity against four different bacterial species, using the cup-plate agar diffusion method. Out of 114 extracts, 83 (73%) exhibited significant activity.

Encarnacion *et al.* (1998) screened the ethanol extracts of 109 plants reported to be used in the traditional medicine of Baja California Sur (Mexico), for antimicrobial activity against: *Candida albicans, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis* and *Bacillus subtilis*. Of these, 64 were active against one or more of the tested organisms.

Firouzi *et al.* (1998) determined the minimal inhibitory concentrations (MIC) of essential oils of five Iranian plants against *Listeria monocytogenes* type four, using a standard tube dilution technique or agar disk diffusion method. In the tube dilution technique, the MIC values for *T. daensis, A. absintium, M. chamommilla, M. officinalis* and *A. sativum* were 1:6400, 1:1280, 1:1280, 1:2560 and 1:1280, respectively. In the agar

disk diffusion test, the zones of inhibition were 25, 9, 10, 10 and 15 mm, respectively.

Frexia *et al.* (1998) screened the dichloromethane and methanol extracts of 19 Latin American plants, for activity against 11 fungal strains. 16 of these plants showed some level of activity.

Jelager *et al.* (1998) evaluated 43 crude drug samples, prepared from 18 plants belonging to 14 families, for antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typi, Candida albicans* and *Aspergillus niger.* 18 showed good activity against the bacteria, and five extracts showed activity against *A. niger.* The antibacterial activities of herbal teas (method by which these plants are used in traditional medicine) made from these plants are presented and discussed.

Hewage *et al.* (1998) screened 101 plant extracts (mostly methanolic) from 55 plants from Sri Lanka, for antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Mycobacterium fortuitum.* Most of the plants showed significant activity against at least one of the tested bacteria.

Iqbal *et al.* (1998) subjected 82 Indian medicinal plants, used traditionally, to preliminary antibacterial screening against several pathogenic and opportunistic microorganisms. Aqueous, hexane and alcoholic extracts of each plant were tested for their antibacterial activity using the agar well diffusion method at sample concentration of 200 mg/ml. The result indicated that out 82 plants, 56 exhibited antibacterial activity against one or more test pathogens.

Lirio *et al.* (1998) screened the aqueous extracts of 36 plant species form Philippines, for antibacterial activity against three bacteria. 21 plant species showed antibacterial activity against all tested pathogens.

Maoz and Neeman (1998) tested the aqueous extracts of 10 plants from Israel including the bark of *Tamarix aphylla*, for their ability to inhibit *T. rubrum, M. canis, Bacillus subtilis, Sarcina lutea* and *Staphylococcus aureus.*

Montes *et al.* (1998) screened the essential oils extracted from flowering aerial parts of four plants from Chile, for activity against strains of eight bacterial species isolated from cured meat and animal feed. All essential oils exhibited a degree of activity.

Omer *et al.* (1998) investigated 66 extracts of 15 Sudanese plants belonging to 12 families for their antibacterial activity against four different bacterial species. Out of 66 extracts, 53 showed significant antibacterial activity.

Sanogo *et al.* (1998) evaluated the antibacterial properties of a decoction and methanol extracts of 13 plant species used in the traditional medicine of Mali, to treat fever and respiratory tract diseases, they were tested for *in vitro* activity against clinically isolated bacterial strains responsible for respiratory infections. The extracts of *Guiera senegalensis* and two other plants exhibited significant activity against all strains of bacteria tested.

Soroush *et al.* (1998) selected crude extracts from 40 Iranian and Canadian plants used in traditional medicine, and tested their activity against several species of *Aspergillus, Candida* and *Cryptococcus*. Of the 40 extracts, 26 (65%) showed activity against at least one fungal strain.

Alasbahi *et al.* (1999) tested the antimicrobial activity of seven plants collected from Yemen, against two Gram positive, two Gram negative bacteria and one yeast, using a qualitative agar diffusion test. All tested plants showed activity against at least one microorganism.

Carvalho *et al.* (1999) screened essential oils of six Brazilian plants used in the popular medicine, for antimicrobial activity against five bacteria and two fungi. The results were variable.

Cavin *et al.* (1999) studied the antifungal activity of 204 crude extracts of Indonesian plants (77 species from 43 families) against *Cladosporium cucumerium* and *Candida albicans*. 20 were active against *Cladosporium cucumerium*, nine against *Candida albicans*.

Choi *et al.* (1999) performed a study to develop antimicrobial substances having various applicabilities as natural preservatives, bio-herbicide agents, medicine, etc. A total of 218 domestic Korean and introduced tree species were screened to identify trees which contain antimicrobial substances against bacteria, fungi and yeasts by the disk-agar plate diffusion method. The antibacterial and antifungal activity was prominent, unlike the anti-yeast activity which was relatively weak.

Facey *et al.* (1999) initiated a systematic scientific study of the medicinal plants, currently used as alternative medicine in Jamaican society, as antimicrobial agents, against five common pathogens. These

studies revealed that 25% of the plant extracts had antimicrobial activity against at least one of the microbes used.

Kudi *et al.* (1999) screened the crude extracts from eight Nigerian medicinal plants (used traditionally to treat infectious and septic diseases) *in vitro* for antibacterial activity, using the hole-plate diffusion method. Most of the extracts were active against Gram positive bacteria. Two of these plants were active against *Escherichia coli* and *Pseudomonas aeruginosa*.

Rajbhandari and Schopke (1999) evaluated the dichloromethane, methanol and 50% aqueous methanol extracts of 13 Nepalese plants for activity against six bacteria and *Candida maltosa* using a modified disk-diffusion method. Growth of *C. maltosa* was only inhibited by the dichloromethane and methanol extract of *Zanthoxylum armatum* (MIC values 500 and 1000μg/disk, respectively).

Sokmen *et al.* (1999) assayed a total of 76 extracts from 35 plants available in the Turkish flora for their *in vitro* antibacterial activities against five pathogenic bacteria and yeast. 16 crude extracts from eight plant species were found to possess an activity against at least one or more test microorganisms. Bioassay-guided fractionation of the most active crude extracts was also carried out with the most active extracts.

Anani *et al.* (2000) investigated the methanol extracts from 19 medicinal plants of Togo, for antiviral and antibiotic activities. 10 of the 19 showed significant antiviral activity and all but two displayed antibiotic activities.

Dutta *et al.* (2000) studied the *in vitro* antifungal activity of *Punica granatum, Psidium gujava, Syzygium cumini* and *Emblica officinalis.* Their leaf and bark tinctures were subjected to antimicrobial sensitivity tests by serial dilution at concentration ranging from 5% to 15% against six test dermatophytes. Bark tinctures exhibited higher efficacy in controlling the mycelial growth of dermatophytes than their respective leaf tinctures. All the tinctures showed fungicidal properties in different concentrations but exhibited only fungistatic property in the case of *Candida albicans.*

Eisa *et al.* (2000) investigated the antimicrobial activities of the chloroform, methanol and aqueous extracts of *Dichrostachys cinerea* fruits and leaves. All tested extracts showed antibacterial activity, the polar ones being the most effective. These results may provide a justification to the traditional use of the plant.

Essawi and Srour (2000) screened the antimicrobial activity of 15 Palestinian medicinal plants (organic and aqueous extracts), against eight different strains of bacteria. Out of the 15 plants, eight showed antibacterial activity. Finally the hole plate diffusion method showed higher activity than the disk diffusion method.

Ishwar and Singh (2000) tested the aqueous and organic solvent extracts of 50 seed plants (from India) belonging to 27 families, for their antifungal activity against *Aspergillus flavus* and *Aspergillus niger* using the agar well diffusion method. Organic solution extracts from 23 species showed antifungal activity.

Jones *et al.* (2000) selected 18 medicinal plants, which were used by the first Nations Peoples of Eastern Canada. Plants were tested for their antifungal activities (against six fungi). Eight randomly selected Tropical plants were also tested for comparative purposes. Disk assays were used. Some of these plants showed significantly fungal inhibiting results.

Ojala *et al.* (2000) performed antimicrobial screening against selected Gram positive and Gram negative bacteria and yeasts, with emphasis on method optimization carried out on methanol extracts prepared from seven plants grown in Finland. Sensitivity to the extracts was found to vary considerably among the microorganisms.

Omer (2000a) screened 44 Sudanese medicinal plants (including *Securidaca longepedunculata*), for their *in vitro* antimicrobial activity against four bacteria and three fungi. The chloroformic and methanolic bark and root extracts of *Securidaca longepedunculata* gave significant results against all tested microorganisms, while the aqueous extracts of the bark and root were inactive.

Rosado *et al.* (2000) studied the methanol and water extracts of six Fabaceae species including *Mimosa pigra*, which are traditionally used in Mayan medicine for the treatment of diarrhea and eye infections, for their *in vitro* antimicrobial activity. *Mimosa pigra* inhibited the growth of *Pseudomonas aeruginosa*.

Vaijayanthimala *et al.* (2000) investigated the anticandidal activity of 20 household South Indian plants against 30 *Candida albicans* isolates obtained from vaginal Candidiasis patients and compared with the anticandidal activity of garlic. Water and ethanol extracts were prepared and their minimum inhibitory concentrations (MICs) were determined.

Some plants exhibited activity in ethanol extracts showing that their active principle is more soluble in a non polar solvent.

Ali *et al.* (2001) screened the antimicrobial activity of 11 *Mimoaceous* plants against various bacteria and fungi and found to be active.

Elegami *et al.* (2001) investigated 99 extracts of 30 Sudanese plants belonging to 19 families for their antibacterial activity against four different bacterial species. Out of 99 extracts, 71 (72%) exhibited significant activity.

Iqbal and Beg (2001) studied the ethanolic extracts of 45 traditionally Indian used medicinal plants for their antimicrobial activity against certain drug resistant bacteria and *Candida albicans* of clinical origin. Of these, 40 plant extracts showed varied levels of antimicrobial activities against one or more test bacteria. Anticandidal activity was detected in 24 plant extracts.

Keles *et al.* (2001) tested the antimicrobial action of the ethanolic extracts from seven Turkish medicinal plants, traditionally used for ailments associated with microbial infections in Livestock. The agar diffusion and dilution methods were used to assess the activity against seven bacteria. All the extracts tested exhibited antibacterial action against *Staphylococcus aureus*, the most susceptible bacterium, with significant differences in action between the different plants. The minimum inhibitory concentration (MIC) of *Hypericum perforatum* showing the highest antibacterial action was 0.12 mg/ml, whereas the MIC values of other extracts ranged from 1 to 4 mg/ml. They concluded that plant extracts with low MIC values may serve as sources for compounds with therapeutic potency.

Khalil *et al.* (2001) subjected the leaves extract of *Combretum aculeatum* Vent, to a preliminary antibacterial screening against two Gram positive bacteria and two Gram negative bacteria. The petroleum ether extract of the leaves was completely inactive towards the organisms tested while methanol and aqueous extracts showed high activity against all tested microorganisms. The active extracts were further tested against 38 clinical isolates, isolated from different sources (Abscess, ear swab, urine and wounds), and their minimum inhibitory concentrations were determined. Two antibiotics were tested against the standard bacteria and the results were compared with the activity of the plant extracts.

Khan (2001) screened the antibacterial activity of acidic, basic and neutral fractions of petrol, ether and chloroform extracts of 39 plant species, collected from the coastal region of Tanzania, with a total of 351 fractions reported. Activity of *Staphylococcus aureus* and *Escherichia coli* was observed in 65 (19%) of the fractions.

Larhsini *et al*. (2001) examined the extracts of 12 Moroccan plants, selected on the basis of folk medicine reports for their antibacterial effects against pathogenic bacteria. The n-butanol extract of *Calotropis procera* flowers and the aqueous extracts of *E. caryophyllata* proved to be the most effective against the bacteria tested.

Srinivasan *et al*. (2001) screened the aqueous extracts of 50 Indian medicinal plants belonging to 26 families, for their antimicrobial activity against 10 bacteria and four fungi. Among the 50 plants tested, 72% exhibited some antimicrobial activity.

Abdelrahim *et al.* (2002) examined the antimicrobial activity of the bark aqueous and methanolic extracts of *Psidium guajava L.*, against four bacteria and clinical isolates. The bark extracts showed a significant antibacterial activity against all tested microorganisms.

Grace *et al.* (2002) tested the stem and fruit of *Kigelia africana* aqueous, ethanol and ethyl acetate extracts, which is a popular source of traditional medicine throughout Africa, for their antibacterial activity, using the microtitre plate assay. All extracts showed significant activity against the tested Gram positive and Gram negative bacteria, supporting the traditional use of the plant in the therapy of bacterial infections.

Omer and Elnima (2002) examined the antimicrobial activity of the methanolic and aqueous bark, leaves, root and stem extracts of *Ximenia americana*, against four bacteria and three fungi. The methanolic extract was the most active one. The aqueous extract also exhibited high activity which justifies the traditional use.

Ijah and Oyebanji (2003) determined the chemical composition of *Enantia chlorantha, Kigelia africana, Bridelia ferruginea, Trema nitems* and *Drypetes gossweileri*, collected from Nigeria, using thin layer chromatography. Alongside this, the activity of the crude aqueous and ethanolic extracts of the medicinal plants species against *E. coli, S. aureus, Ps.aeruginosa, Klebsiella sp.* and *Proteus sp.* causing urinary tract infection was determined. The extracts of the medicinal plant species inhibited the growth of the bacteria tested.

Silva and Gomes (2003) isolated a new compound from the leaves of *Guiera senegalensis*, which exhibited potent antifungal activity against *Cladosporium cucumerinum*.

Hanan *et al.* (2004) tested the volatile oil, gum and resin ethanolic extracts of *Pistacia lentiscus L.* (Misteka), 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.

1.2.2. Wound Healing activity of Medicinal plants:

The use of Medicinal plants as wound healing agents is very well documented.

Pal and Gupta (1979) studied the *in vivo* antifungal activity of "*Choti dudhi* plant" (*Euphorbia prostrata Ait.* and *Euphorbia thymifolia Linn.*) against certain dermatophytes, using experimentally infected goats and rabbits. The animals were inoculated with cultures of *Trichophyton mentagrophytes* or *T. simii* and comparable lesions were left untreated/treated with benzoic acid in linseed oil or treated with an ether extract, in linseed oil, of powdered *Choti dudhi*. The plant extract cured the lesions in 20-33 days and was as effective as benzoic acid, which cured lesions in 24 days.

Das *et al.* (1989) reported significant anti-inflammatory activity along with moderate antibacterial and antifungal activities in the alcoholic extract of the seed kernel of *Mangifera indica*, where Male albino rats were used (120-200 g). Their observations proved the validity of the ethnic use of the seed kernel against minor cuts and burns. The presence of both antibacterial and anti-inflammatory activities in the test substance is suggestive of its use in rheumatic diseases where bacterial invasion may be an etiological factor in causing inflammation.

Sarma and Aithal (1990) investigated the anti-inflammatory and wound healing activities of the crude alcoholic extract and flavonoids of *Vitex leucoxylon*. In acute inflammation model the crude aqueous extract as well as the mixture of flavonoids of *Vitex leucoxylon* showed anti-inflammatory activity without any effect on chronic inflammation. The crude extract significantly reduced the wound breaking strength. Albino rats of either sex were used (150-250 g) individually housed with water *ad libitum* (as much as one likes). Wound healing activity was studied on three different experimental wound models: incision, excision and dead space.

Caceres *et al.* (1991) reported the result of *in vitro* bioassays of 30 plant species from Guatemala, in order to validate the effects of their traditionally used extracts against *Candida albicans* which causes dermatomucosal infections. Six of these plants showed anticandidal activity. The most active were *B. crassiflora* and *P. guajava*

Brantner and Grein (1994) screened the aqueous extracts of 31 Austrian plants (from 28 families), selected on the basis of medicinal folklore use

(for external treatment of infected skin lesions) and literature data, for their antibacterial activity against *Bacillus subtilis, Escherichia coli, Enterococcus faecalis*, and two strains of *Stapylococcus aureus*. The results indicated that about 60% of the plant extracts tested exhibited some level of antibacterial action.

Udupa (1994) reported the anti-inflammatory and wound healing properties of *Aloe vera*. The fresh juice of the indigenous drug *A. vera* (0.2 ml/100g, i.p.) was studied for its anti-inflammatory and wound healing properties in rats. Anti-inflammatory action was studied by observing percent reduction in carrageenin-induced paw odema at 3h. Wound healing effects were studied on incision (skin breaking strength), excision (percent wound contraction and epithelisation time) and dead space (granuloma breaking strength and biochemical parameters) wound models. *Aloe vera* showed significant anti-inflammatory activity in acute inflammatory model without any significant effect on chronic inflammation. Significant increase in breaking strength (skin and granuloma tissue), enhanced wound contraction and decreased epithelisation period were observed. This drug could therefore increase tensile strength by increasing cross-linking in collagen and interactions with the ground substance.

Vedros and Steinberg (1994) examined the *in vitro* and *in vivo* activity of plant extracts for use on canine pyotraumatic dermatitis. An isolated algal extract and extracts selected for antimicrobial activity were used on one case of three dogs and one cat with pyotraumatic dermatitis. The medicinal plant extracts relieved the symptoms of pain and inflammation and in combination with *Licorice* root (*Glycyrrhiza glabra var. typical*) extract, condensed tannins, pomacepectin and camphor to be bactericidal as well as fungicidal and hence provide temporary treatment of pyotraumatic dermatitis.

Ahmed *et al.* (1995) examined the effect of some medicinal plant extracts on wound healing in farm animals. Experimental wounds were made in six groups of five calves, five goats and five sheep. Two wounds were made in each animal, one wound was used as a control and the other was treated topically with either *Matricaria chamomilla* (*Chamomilla recutita*) lotion and ointment; *Salix fragilis* lotion; *M. chamomilla* lotion and *Polygonum bistorta* ointment; *S. fragilis* lotion and *P. bistorta* ointment; *Nigella sativa* lotion or left as a control. Clinical, histopathological, histochemical and microbial studies showed that healing was best with *M. chamomilla* lotion, followed by both of

M. chamomilla lotion and *P. bistorta* ointment, *N. sativa* lotion, *S. fragilis* lotion and *P. bistorta* ointment, with *S. fragilis* lotion being the least effective.

Tandan *et al.* (1995) evaluated *Azadirachta indica* seed oil, for its wound healing activity. It was given orally to mice and showed low toxicity. It was non-irritant to the skin of rabbits in primary dermal irritation test. The study suggested that *A. indica* oil is devoid of any adverse effect on kidneys and liver, is non-irritant to the skin of rabbits and is, therefore, relatively safe for external application in wounds.

Bosisio *et al.* (1997) studied the biological activity of *Guiera senegalensis*. Its leaves are used in African traditional medicine for gastrointestinal disorders, coughs and topically for wound healing. The antimicrobial activity of the leaf extracts were examined against Gram positive, Gram negative bacteria, moulds and yeasts. The crude extract possessed a mild antimicrobial effect only on Gram positive bacteria (MIC 0.8-1.5 mg/ml).

Lentz *et al.* (1998) reported 92 plants used in the traditional pharmacopoeia of the Pech and neighboring Mestizo peoples of central Honduras, for their *in vitro* antimicrobial activity. 19 of the extracts showed signs of antifungal activity while 22 demonstrated a measurable inhibitory effect on one or more bacterial cultures. The broad spectrum of activity of the extracts helped to explain the widespread of these plants for wounds and other applications.

Mohtar *et al.* (1998) tested 38 species of Malaysian medicinal plants for their antimicrobial activity against several pathogenic micro-organisms causing skin infections in Man. The plant extracts were subjected to a modified disk-diffusion technique whereby antimicrobial activity was evaluated based on the ability of the plant extracts to diffuse through the agar to affect the target microorganism. The antimicrobial activity of some of these plants was very high.

Amani *et al.* (1999) investigated the aqueous and alcoholic extract of 15 plant species; all used in folk medicine in Argentina to heal wounds, for their antibacterial activity against eight different bacteria. The extracts exhibited different potencies of activity against Gram positive and Gram negative bacteria.

Grierson and Afolayan (1999) examined the antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern

Cape, South Africa. Ethnomedical information gathered from surveys at clinics, hospitals as well as interviews with traditional healers and rural dwellers revealed that *Grewia occidentalis, Polystichum pungens, Cheilanthes viridis* and *Malva parviflora* are the most commonly used plants for the treatment of wounds in the province. The methanolic extracts of *Grewia occidentalis, Polystichum pungens* and *Cheilanthes viridis* showed significant inhibitory activity against Gram positive and Gram negative bacteria, while the acetone extract of *Polystichum pungens* inhibited the Gram negative bacteria only. Extracts from *Malva parviflora* did not show any antibacterial activity at 5.0 mg/ml.

Shale *et al.* (1999) screened the medicinal plants used in Lesotho for antibacterial and anti-inflammatory activity. As a result of ethnobotanical data obtained, leaves and roots of 16 plants were extracted using hexane, methanol and water, and the respective extracts screened for antibacterial activity using the disk-diffusion assay. Six species displayed very high antibacterial activity against both Gram positive and Gram negative bacteria. The activity was mainly found in the root.

Ali *et al.* (2001) carried out an Ethnobotanical survey in the Palestinian area, to evaluate the relative efficacy of the plants used to treat skin diseases and prostate cancer. 59 plants were claimed to be effective.

Portillo *et al.* (2001) *in vitro* assayed the antifungal activity of aqueous, dichloromethane and methanol extracts from 14 Paraguayan plants, used in traditional medicine for the treatment of skin diseases, against 10 fungi. Among them, dichloromethane extracts of four plants, as well as aqueous and methanol extracts of one plant, showed the highest activity.

Arzi *et al.* (2003) studied the wound healing stimulation activity of Licorice "*Glycyrrhiza glabra*" in rabbits. *Glycyrrhiza glabra* is one of the most widely used medicinal plants and is employed in numerous traditional and modern preparations. In this study the healing effect of Licorice extract was investigated on open skin wound in rabbits. Newzealand rabbits of either sex weighing 1.8-2.2 kg were used during the study. Hair of lower back and left flank of animal were completely shaved. Full thickness wound (20x20mm) was made on the shaved area. Hydroalcoholic extract of Licorice was prepared and applied two times daily. Phenytoin cream 1% was used as standard control. Treated groups were compared with non-treated groups. Healing was determined by reduction in wound area. The results of this study confirmed that licorice cream 1% is a potent healing agent even better than phenytoin.

1.2.3. Principles of Anti-infective Therapy:

Modern antimicrobial chemotherapy developed in three Eras. The first era (1600-1900 A.D.) involved the use of an extract of the bark of the *Cinchona* tree to treat malaria successfully; the active principle of *Cinchona* bark, Quinine, was first isolated in 1820 and is still in use till today.

The second Era is that of synthetic compounds which began around (1900 A.D.) by the German Chemist Ehrlich, whose work laid the foundation upon which all antimicrobial chemotherapy is built. He was the first to coin the word chemotherapy to describe his work.

The third Era came in (1935 A.D.), when Domagk in Germany began work on the effect of a number of dyes on mice infected with *Streptococci*. One compound, Prontosil, was effective. It was the first compound of Sulphonamides, it gave Sulphanilamide after hydrolysis (Edwards, 1980).

An Antibiotic is a naturally occurring product of one organism that is inhibitory to others.

According to this definition, chemical compounds such as Sulphonamides, Quinolones, Nitrofurans and Imidazoles should be referred to as chemotherapeutic agents.

However, some antibiotics can be manufactured synthetically, while others are the products of chemical manipulation of naturally occurring antibiotics 'semi-synthetic antibiotics' (Greenwood *et al*, 1994).

Cheesbrough (2000) defined Antibiotics as antimicrobial agents (usually antibacterial) that can be used to treat infection.

A Bacteriostatic is having the property of inhibiting bacterial multiplication; which resumes upon removal of the agent.

A Bactericidal is having the property of killing bacteria. Bactericidal action differs from bacteriostatic only in being irreversible; i.e. the killed organism can no longer reproduce, even after being removed from contact with the agent.

Selective toxicity is mentioned when an ideal antimicrobial agent exhibits selective toxicity. This term implies that a drug is harmful to a parasite without being harmful to the host.

The chemotherapeutic index (selective toxicity) compares the maximum dose that can be tolerated by the host without causing death, with minimum dose that cures the particular infection.

$$\text{Chemotherapeutic index} = \frac{\text{maximum tolerated dose}}{\text{minimum curative dose}}$$

(Omer, 2000)

1.2.4. *In vitro* Antimicrobial activity:

Antimicrobial activity is measured *in vitro* in order to determine:

1- The potency of an antimicrobial agent in solution. Sensitivity tests are also used to evaluate new antimicrobial agents by testing them against large number of organisms of known susceptibility and to compare these results with drugs already available (Garrod *et al*, 1981).

2- Drug concentration in body fluids or tissue.

3- The sensitivity of a given microorganism to known concentration of the drug.

1.2.4.1 Measurement of antimicrobial activity:

Determination of the drug quantity may be undertaken by one of two methods: dilution or diffusion. Using an appropriate standard test organism, these methods can be employed to estimate either the potency of antibiotic in the sample or the sensitivity of the microorganism.

1.2.4.1.1 Dilution methods:

The most commonly used method in the dilution techniques is the broth dilution technique, first described by Rammelkamp and Maxon (1942). This involves exposing bacteria to increasing concentrations of antimicrobial agents in liquid media, usually by serial dilution. The system consisting of an organism, a tested drug and a nutrient medium, is then incubated at 37°C for 18-24 hrs., and the end point at which no

visible growth occurs (the first tube showing no turbidity) is defined as the MIC, bactericidal end points may be obtained at intervals from the same system by subculturing known volumes from each tube on solid media under conditions in which the drug transferred will be destroyed, antagonized or diffused away from viable organisms (Anderson, 1970; Garrod *et al*, 1981).

Dilution tests on solid media involve addition of varying concentrations of drug to measured volumes of agar medium which has been melted and cooled to 45-50°C; the resultant mixtures are then poured as plates into Petri dishes or as slants into test tubes. Standardized inocula are seeded onto the surface of the medium and MIC read after an appropriate incubation period (Garrod *et al*, 1981). In these methods, it is essential to test strains of known susceptibility with each series of unknowns in order to minimize error due to drug deterioration, inaccuracies in dilution or variation in the medium (Cruickshank e*t al*, 1975).

Break point concentrations of antibiotics are used to characterize antibiotic activity: the interpretive categories are active, moderately active (intermediate), and inactive. These concentrations are determined by considering pharmacokinetics, serum and tissue concentrations following normal dose, and the population distribution of MICs of a group of bacteria for a given drug.

1.2.4.1.2 Diffusion methods:

Diffusion test is less expensive but less reliable than the dilution method; however, it provides qualitative susceptibility information.

In the diffusion technique, the organisms to be tested is exposed on an agar medium, either seeded in the agar, by flooding or spreading, to a diffusion gradient of the chemotherapeutic drug arising from a reservoir, which may be impregnated filter paper disk, ditch cut in the medium and filled with (agar containing) the drug, or the ditch may be filled directly with drug solution, or the solutions of the drugs may be placed in circular holes cut in the inoculated agar medium (Anderson, 1970; Garrod *et al,* 1981). The reservoir may also be a compressed tablet (Lund *et al*, 1951).

1.2.4.2. Factors affecting the *In vitro* antimicrobial activity:

  The diffusion process may be altered by a variety of factors including:

1- The content of drug at the source, the density of the gel, the speed of diffusion and the ionic concentration of the medium (Kavanagh, 1963; 1972).

2- The zone of inhibition that results is dependent upon both the diffusibility of the agent in the medium and the degree of susceptibility of the organism. The speed of growth and the size of inoculum can influence to a marked degree the size of inhibitory zones (Peter and Plorde, 1963; Cooper, 1963; 1972).

3- The pH of the environment also affects antimicrobial activity *in vitro*. Some drugs are more active at acid pH (e.g. nitrofurantoin); others at alkaline pH (e.g. aminoglycosidses, sulphonamides), (Omer, 2000).

4- The components of the medium significantly influence the results of the tests e.g. sodium polyanethol sulphonate and other anionic detergents inhibit aminoglycosides. PABA in tissue extracts antagonizes sulphonamides. Serum protiens bind penicillin in varying degrees, ranging from 40% for methicillin to 98% for dicloxacillin (Omer, 2000).

5- The stability of drugs at incubator temperature. Several antimicrobial agents lose their activity. Chlortetracycline is inactivated rapidly and penicillins more slowly, whereas aminoglycosides, chloramphenicol and ciprofloxacin are quite stable for long periods (Omer, 2000).

6- Length of incubation: In many instances, microorganisms are not killed but only inhibited upon short exposure to antimicrobial agents. The longer the incubation continues, the greater the chance for resistant mutants to emerge or for the least susceptible number of the microbial population to begin multiplying as the drug deteriorates (Jawetz *et al*, 1995).

1.2.5. *In vivo* Antimicrobial activity:

  In vivo methods involve determination of the amount of drug or derivatives in blood, urine, faeces or tissues at intervals after rubbing a defined amount of material into the skin under standard conditions (Shayoub, 1985).

1.2.5.1. Selection of the appropriate Laboratory animals:

Animals are used to show standard response to experimental manipulation. Animals must be of the same: species, subspecies, strain, 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, 1989).

1.2.5.2. Skin:

Skin has many essential functions including protection, thermoregulation, immune responsiveness, biochemical synthesis, sensory detection and social & sexual communication.

Therapy to correct dysfunction in many of these activities may be delivered: systematically, intralesionally, topically and through ultra-violet radiation. Topical therapy is a convenient method of treatment (Hardman *et al*, 1996).

1.2.5.2.1. Normal wound healing:

Normal wound healing is a process that is allowed to proceed without undue interference from infection, excessive tissue devitalization, poor wound repair technique, underlying patient conditions and diseases and inhibitory drugs (Trott, 1985).

Healing of wound involves various phases such as inflammation, coagulation, collagenation, wound contraction and epithalization, While the phases between coagulation and collagenation are interlinked, the phases of wound contraction and epithalization are completely independent from each other but run concurrently (Bairy and Rao, 2001).

1.2.5.2.2. Skin diseases:

Diseases of the skin may be primary or secondary in origin; in primary skin diseases the lesions are restricted initially to the skin although they may be spread from the skin to involve other organs. On the other hand,

cutaneous lesions may be secondary to disease originating in other organs (Blood, 1983).

1.2.5.2.2.1. Definition of wound infection:

Precisely defining clinical wound infections is something difficult to do. Normal inflammation and infection have similar characteristic findings like: warmth, redness, tenderness, and swelling. Infection can be identified by the greater degree to which these signs are exhibited. An infected wound is often subjectively painful to the patient and very tender to palpation by an examiner. A non infected wound is usually only mildly painful, if at all (Trott, 1985).

1.2.5.2.2.2. Bacterial pathogens causing wound infection:

The following bacteria cause wound infections:

Staphylococcus aureus, Escherichia coli, Proteus species, Klebsiella species, Enterococcus species, Pseudomonas aeruginosa, Clostridium perfringens, Bacteriodes fragilis, Anaerobic cocci (Cheesbrough, 2000).

Staphylococcus aureus is the most common causative of wound infections (Gosnold, 1977).

1.2.5.3. Utilization of plants for wound healing:

The use of crude drugs undoubtedly dates back long before recorded history, for the instinct of primitive Man to relieve the pain of a wound by bathing it in cool water or by soothing it with fresh leaf or protecting it with mud is within the realm of belief (Ansel *et al*, 2000).

In Sudan, many plants, including *Solenostemma argel* leaves, are used traditionally for healing of inflamed wounds (Elkamali, 1997).

Shayoub, (2003) has listed the uses of *Solenostemma argel* in folkloric medicine in Sudan, and one of them is externally in the form of poultice as anti-inflammatory.

The success of antimicrobial therapy depends upon maintaining at the site of infection a drug concentration that will result directly or indirectly in the death or control of the infectious organisms with minimal deleterious effect to the host. In order to achieve this aim the antibacterial agent must have activity against the organism at its site of infection and it

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must be administered in such a way as to maintain an effective inhibitory or lethal concentration (Blood, 1983).

In literature surveyed, no previous studies on the *in vivo* antimicrobial and wound healing effect of *Solenostemma argel* have been done, and regarding its traditional uses, *Solenostemma argel* leaves will be subjected to *in vitro* and *in vivo* study to validate these claims.

1.2.6. Ointments:

Ointments are semisolid preparations intended for external application to the skin or mucous membranes. 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 vehicle in the preparation of medicated ointments (Ansel *et al*, 2000).

1.2.6.1. Ointment bases:

Ointment bases are classified by the USP into four general groups:

i) Hydrocarbon bases.

ii) Absorption bases.

iii) Water removable bases.

iv) Water soluble bases.

1.2.6.1.1. Hydrocarbon bases:

Also termed *Oleaginous* bases. On application to the skin:

i) They have an emollient effect.

ii) Protect against the escape of moisture.

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 preparations 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.

1.2.6.1.1.1. 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° 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).

1.2.6.1.1.2. Liquid paraffin:

This 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 (Collett and Aulton, 1991).

1.2.6.1.2. Absorption bases:

They are of 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*, 2000).

1.2.6.1.2.1. 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.

1.2.6.1.3. Water removable bases (water miscible):

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

ii) Because the external phase of the emulsion is acqueous, 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 base (Ansel *et al*, 2000).

The three emulsifying ointments form water-miscible bases, i.e. Emulsifying 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).

1.2.6.1.4. Water soluble bases:

It do not contain oleaginous components. They are completely water washable and often referred to as 'Greaseless' because they soften greatly with the 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. Polyethylene Glycol ointment, NF is the prototype example of water soluble base.

1.2.6.1.4.1. Polyethylene Glycol ointment, NF (macrogol or carbowaxes):

Polyethylene Glycol (PEG) is a polymer of ethylene oxide and water presented by the formulation H $(OCH_2CH_2)_n$ OH in which n represents the average number of oxyethylene groups. The numerical designations associated with PEGs refer to the average molecular weights of the polymer. PEGs having average molecular weights below 600 are clear, 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 NF lists the viscosities of PEGs ranging from average molecular weights of 200 to 8000. The general formula for the preparation of 1000g of Polyethylene glycol ointment is:

Polyethylene glycol 3350   400grams
Polyethylene glycol 400   600grams

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

1.2.6.1.4.1.1. Advantages of Polyethylene Glycol:

1/ PEGs vehicles form non occlusive films on skin.

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

3/ Good absorption by the skin.

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

5/ Freedom from greasiness.

6/ Satisfactory ageing properties. They do not hydrolyzse, rancidify or support microbial growth.

7/ Compatibility with many dermatological medicaments e.g. ammoniated mercury, yellow mercuric oxide, Icthammol and Sulphur (Robinson *et al*, 1964).

1.2.6.1.4.1.2. Disadvantages of Polyethylene Glycol:

1/ Limited uptake of water, macrogols dissolve when the proportion of water reaches about 5%, bases may be thinned with liquid macrogol or with propylene glycol. Inclusion of a higher fatty alcohol, such as cetostearyl, which allows incorporation of larger quantities 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 quaternary ammonium compounds (Patel and Foss, 1964). And some antibiotics are rapidly inactivated such as penicillin and bacitracin (Coutes *et al*, 1961).

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

1.2.6.2. 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 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 a low 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).

1.2.6.3. Selection of the appropriate base:

The selection of the base to use in the formulation of an ointment depends upon the careful assessment of a number of factors including:

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 a base that 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).

1.2.6.4. 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.

1.2.6.4.1. Mixing by fusion:

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

1.2.6.4.1.1. Preparation 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

ingredients, such as hard paraffin and the emulsifying waxes, are grated into the basin and heated while other ingredients are being prepared.

After melting, the ingredients should be stirred gently until cool.

1.2.6.4.1.2. Preparation of Medicated Ointments and Pastes by Fusion:

Solids that are completely or partially soluble in the base should be added in fine powder to the molten base at as low a temperature as possible and the mixture stirred until cold (Collett and Aulton, 1991).

1.2.6.5. Application frequency:

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

1.2.6.6. Microbial contents:

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

1.2.7. Botanical, Ethnopharmacological and Phytochemical profiles of the studied medicinal plants:

1.2.7.1. *Raphanus sativus* Linn.

Common name: Radish, Garden Radish (Saad *et al*, 1988).

Vernacular name: Figil (Arab.).

Family: Cruciferae (Andrews, 1950).

Botanical description: Radical leaves lyrate, flowers white, yellow or lilac. Siliqua 1-2 inch long, terete, beaked (Broun and Massey, 1929).

Habitat: Light soil.

Distribution: Cultivated everywhere in Sudan (Broun and Massey, 1929), and world-wide (Saad *et al*, 1988).

Chemical constituents: Saad *et al*, (1988) reported that roots contain Raphaiol, Rettichol, Sinapine and Rhaphanin 'the later posses antibacterial activity'. Seeds contain volatile and fatty oil, and Roots are rich with Ca, Fe and Vit.C.

Terras *et al*, (1992) analyzed two novel homologous, 5-KD cysteine rich antifungal proteins from the seeds, designated: *Raphanus sativus* antifungal protein 1 and *Raphanus sativus* antifungal protein 2 (Rs-AFP1 & Rs-AFP2).

Folkloric use: used for treatment of acne. Broun and Massey (1929) reported that the seeds, which yield oil, are used in native medicine for their diuretic and laxative properties, the seed pods as well as the roots are eaten. Boulos, (1983) reported that the plant is an appetizer, stimulant, fresh roots digestant, diuretic, antiscorbutic, dried root diuretic, decoction of leaves antiphlogistic, seeds stomachic, expectorant.

1.2.7.2. *Tamarix aphylla* (Linn.) Karst.

Common name: Tamarisk.

Vernacular name: Tarfa (Arab.).

Family: Tamaricaeae.

Botanical description: Trees up to 15m high with a girth of about 2-4 m. Bark reddish brown to grey. Longitudinally fissured branches, apparently articulated, usually slender, green, hoary with dusty grey glandular efforescence and closely jointed, larger branches with persistent scale like leaves. Leaves reduced to minute triangular teeth on a sheathing base. Flowers subsessile, rose about 3 x 1.5 mm (Elamin, 1990).

Habitat: Along river banks and water courses on light silty soils (Elamin, 1990).

Distribution: Widely all over Sudan (Elghazali, 1997), especially along the Gash delta, Kor baraka and Arbaat (Red sea hills), and on the banks of the Blue and White River Nile (Elamin, 1990).

Chemical constituents: Polyphenolics, Tamarix ellagic acid and Ellagotannin (Elghazali *et al*, 1998).

Folkloric use: Has been mentioned as a tree of medical value in Quran and Bible. The Plant is astringent, used for oedema of spleen, enteritis, gastrelgia, and diarrhea, and also it is used in the form of stabilized preparation for ulcerating piles and anal fissures (Boulos, 1983).

 Elghazali *et al*, (1998) reported that the bark is used traditionally for treatment of hemorrhoids. Saad *et al*, (1988) reported that the branches are used in syphilis and skin diseases, diarrhea and colic.

1.2.7.3. *Solenostemma argel* (Del.) Hayne.

Vernacular name: Hargel (Arab.).

Family: Asclepiadaceae (Elghazali et al, 1998).

Botanical description: Shrub 8-12 ft. high. Stems pale grey green, minutely puberulous. Leaves pale-grey-green, lanceolate, obtuse to subacuminate at the apex, ¾ - 1 ½ inch long, minutely puberulous. Flowers white, numerous in axillary cymes. Follicles ovoid-lanceolate, tapering to a blunt apex, 2 ½ - 4 inch long, glabrous (Andrews, 1952).

Habitat: Light sandy soil to heavy clay (Elkamali, 1997).

Distribution: Cultivated in north and central parts of Sudan (Elkamali, 1997).

Chemical constituents: Mahran *et al*, (1976) reported the isolation of Argelin and Argeloside. Khalid (1974) detected the presence of flavonoides, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids in *S. argel*. Khalid *et al*, (1974) reported the isolation of kaempferol, its mono and di-glycosides and querctin from the leaves and stems of *S. argel* of Sudanese origin. Elkamali, (1997) reported the presence of protein, sugars, fiber, vitamins and minerals (Na^+, K^+, Ca^{++}, Ni^{+3}, Mg^{+2} and P^{+3}). Kamel, (2003) isolated four new acylated phenolic glycosides from the aerial parts of *S. argel*. Hamed, (2001) isolated two new pregnane ester glycosides named stemmoside A (1) and stemmoside B (2), and a third new polyhydroxy pregnane named stemmin C (3), from the leaves of *S. argel*.

Folkloric use: used traditionally for colic, digestion disorders, diabetes, nephritis and measles (Elghazali *et al*, 1998). Also used as diuretic (Saad *et al*, 1988), and as spasmolytic, pain killer, colic's accompanying dysmenorrhea (Eltahir *et al*, 1987). Elkamali, (1997) reported that the alcoholic extract of stems has got antifungal activity, antispasmodic effect in the leaves aqueous extract (uterine muscle relaxant) for the presence of flavonoides. The decoction of leaves and stems is used in Sudan, Egypt, Libya, Sudia Arabia and Algeria for coughs, influenza, intestinal disorders and stomach aches. Leaves have diuretic properties, expectorant, antipyretic, digestant, for urinary infections, syphilis. Fine powdered leaves are used for treatment of infected wounds, fumigation of leaves for measles, in Libya and Chad the boiling leaves for neuralgia and sciatica.

1.2.7.4. *Mimosa pigra* Linn.

Synonymus name: *Mimosa asperata* L.

Common name: Thorn blood; Sensitive plant.

Vernacular name: Shajarat Elfas, Alset almostehia (Arab.).

Family: Mimosaceae.

Botanical description: Prickly shrubby plant, branchless and leaf-rachis prickly and roughly hirsute. Pinnate in 7-16 pairs, arranged along the prickly leaf rachis. Flower heads pale mauve, globose on axillary peduncles about 1 inch long. Pod densely bristly all over, 1½ -2½ inches long. Segments narrow and numerous (Andrews, 1952).

Habitat: Often forming dense thickness on river banks.

Distribution: Wide-spread all over Sudan (Andrews, 1952).

Chemical constituents: Yusuf *et al,* (2003) reported the presence of flavonoids glycosides in the leaves of Mimosa species.

Folkloric use: Leaves are used traditionally for eczema. Adjanohoun *et al*, (1991) reported that it is used traditionally for the treatment of children febrile convulsions.
 It is used in the Mayan traditional medicine (Yactan) for the treatment of diarrhea and eye infections (Vallado *et al*, 2000).

1.2.7.5. *Guiera senegalensis* J.F. Gmel., Linn.

Vernacular name: Ghubeish (Arab.).

Family: Combretaceae (Elghazali, 1997).

Botanical description: Grey tomentose shrubs up to 2 m high, leaves opposite or subopposite, elliptic-oblong, 6-12 x 0.5-2.8 cm, apex mucronate base slightly cordate to attenuate; margin entire (Elghazali *et al*, 1987).

Habitat: Lowland sandy plains, degraded savanna.

Distribution: Rashad, Wad ashana and central Sudan.

Chemical constituents: Flavonoids, saponins, alkaloids, mucilages and tannins (Elghazali, 1997).

Folkloric use: Leaves used as antidiabetic, antipyretic, anti-vomiting and antileprosy (Elghazali *et al*, 1987). Also leaves in African traditional medicine for gastrointestinal disorders, coughs and topically for wound healing (Bosisio *et al*, 1997).
Plant is used for Malaria in Mali and Sao-tome traditional medicine (Ancolio *et al*, 2002).

1.2.7.6. *Tinospora bakis* (A. Rich) Miers.

Synonomus name: *Cocculus bakis* (A. Rich)

Vernacular name: Irg al hagar (Arab.), Mama (Fung).

Family: Menispermaceae.

Botanical description: Slender glabrous lianes. Branchlets with lenticels, leaves broadly ovate-triangular, 5-8 cm across thinly papery, glabrous, digitately 7 nerved at base, petiole 2.5-8.8 cm long, slender.

Habitat: Lowland plains.

Distribution: Komul (Ingassana area, Sudan).

Chemical constituents: Palamtine isoquinoline alkaloid was isolated (Elghazali *et al*, 2003).

Folkloric use: The maceration of the roots is used for headaches (Elghazali *et al*, 2003).

1.2.7.7. *Xeromphis nilotica* (stapf) keay, Bull.

Synonymus name: *Randia nilotica* (stapf) J. Linn. Soc. Bot.
       Catunaregam nilotica
       Lachnosiphonium niloticum (stapf) Dand ex F.W.

Vernacular name: Shgar-Elmarfien, Umm takirni (Arab.)

Family: Rubiaceae.

Botanical description: Tomentose, deciduous many stemmed shrubs, with stiff spines. Leaves in clusters below the spines, sessile, obovate, 1.6-3.5 x 0.8-2 cm, apex rounded, base cuneate and margin entire. Fruit drupes, globose, 1.5-1.7 cm across, grey.

Habitat: Lowland plains.

Distribution: Widespread especially around Rashad.

Chemical constituents: The bark afforded a range of hydrocarbons, sterols, triterpenes both free and esterified fatty acids. Mannitol, scopoletin, scopolin, umbelliferone, iso-scopoletin and synergic acid were reported from methanol extract of the bark. Oleanolic acid, randialic acid and quinovic acid were also isolated (Elghazali *et al*, 1987).

Folkloric use: The water extract of the bark is used as an anthelmintic, for jaundice and for Rabies (Elghazali *et al*, 1987).

1.2.7.8. *Securidaca longepedunculata* Fresen., Mus. Senk.

Common name: Violet tree.

Vernacular name: Irg-Alali (Arab.), Sowa (Fung).

Family: Polygalaceae.

Botanical description: Small trees, up to 5 m high, barks smooth pale, leaves simple, alternate, oblong lanceolate, c. 5.3 x 0.9 cm, glabrous. Fruits samaras, yellow brownish, 4.0-1.4cm with oblong wings, nuts containing the seeds rugulose, c. 1 cm across.

Habitat: Rocky lands at high elevation.

Distribution: Gebel Azole (Ingassana area).

Chemical constituents: Alkaloid mixture. Indole alkaloids phenyporaponids were reported (Elghazali *et al*, 2003).

Folkloric use: The maceration of the bark is used to treat stomach pains, headaches and tooth pain (Elghazali *et al*, 2003). Also as anti-dote against venomous snake bites and to treat blennorrhagia and helminthiasis (Adjanohoun *et al*, 1991).

1.2.7.9. *Kigelia africana* (Lam.) Benth.

Synonymus name: *Bignonia africana* (Lam.) Encycl.
Kigelia aethiopica (Decne) Del.
Kigelia aethiopium (Fenzl) Dandy.

Common name: Sausage tree.

Vernacular name: Abu-Shutour, Umm Mashtour (Arab.).

Family: Bignoniaceae.

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 x 1.8-3 cm, apex rounded to mucronate. Fruit berres, 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 acid were isolated from the bark. Different morphological parts of *K. pinnata* were shown to contain dihydro-isocoumarins, 6-methoxy mellin, an iridoid glucoside (veratraldehyde) terpenoid aldehyde and napthaquinone (Elghazali *et al*, 1987).

Folkloric use: Traditionally used in West Africa for wounds and abscesses. The water extract of the bark is used for backache, stomach pains and dysentery (Elghazali *et al*, 1987). Plant is used as antimalaria, febrile jaundice, menorrhagia, impending abortion and women sterility (Adjanohoun *et al*, 1991). Leaf for snake bite; soften wounds; fruit for constipation, piles, female disorders, as purgative; females rub breasts with fruit bulb as a cosmotic (Ayensu, 1978).

1.2.7.10. *Courbonia virgata* brongn.

Vernacular name: Kordala (Arab.).

Family: Capparidaceae.

Botanical description: Glabrous glaucous small shrub, with tuberous root and twiggy leafy branches, leaves narrowly elliptic or lanceolate, acute at the apex, coriaceous, ¾ - 1¼ in. long up to ½ inch. Flowers numerous, axillary, solitary. Fruit globose, about 1 cm in diameter (Elghazali, 1986).

Habitat: Clay soil.

Distribution: Nuba mountains, Red sea, Berber, Blue and White Nile, Fung, Khordofan and Bahr Elgazal province (generally distributed).

Chemical constituents: Quaternary ammonium compounds were detected in the Capparaceae (Mclean *et al*, 1996).

Folkloric use: Ash of the leaves and stem used as substitute for salts, fruits are edible (Elkhatib and Abdalla, 2002).

CHAPTER TWO

MATERIALS
&
METHODS

CHAPTER TWO

MATERIALS & METHODS

2.1. MATERIALS:

2.1.1. Chemicals and Reagents:

Amyl Alcohol	The British Drug House, England
Chloroform AR	S.D. Fine-Chem. Ltd., Mumbai
Crystal violet	The British Drug House, England
D (-) Glucose anhydrous	Park, Scientific Ltd., UK
D (+) Lactose	Park, Scientific Ltd., UK
D (-) Mannitol extra pure	Kiran Light Lab.
Ethylenediamine tetra-acetic acid	The British Drug House, England
Emulsifying wax	Medex, England
Halothane	ICI Ltd., India
Hydrochloric acid	The British Drug House, England
Hydrogen peroxide	Bell, sons & Co., England
Immersion oil	The British Drug House, England
Iodine	Hopkins & Williams Ltd.
Kovac's reagent	B.G.H. Chemicals, Japan
Lactophenol cotton blue	The British Drug House, England
Lanoline B.P.	Sukani Enterprises, India
Liquid paraffin BP	Bell, sons & Co., England

Methanol   Scharlu, Spain

Methyl Red   Oxoid, Ltd.

Oxidase   John baker Inc., USA

Peptone   Oxoid, Ltd.

Petroleum Jelly B.P.   Bogdany, Hungary

Phenol Red   The British Drug House, England

Polyethylene glycol (PEG)   Fluka AG Chemische Fabrik

Safranin Red   The British Drug House, England

Sodium Chloride   The British Drug House, England

Sucrose AR   S.D. Fine-Chem. Ltd., Mumbai

Urea   Oxoid, Ltd.

2.1.2. Chemotherapeutic agents:

Ampicillin   Aurobindos Pharma Ltd., India
                                  Expairy date: August 2006

Clotrimazole   Shin Poong Ph. Co.Ltd., S. Korea
                                  Expairy date: May 2005

Cloxacillin   Beecham Pharmaceuticals
                                  Expairy date: November 2005

Gentamicin   SPIC, China
                                  Expairy date: March 2006

Nystatin   Sigma Chemical Co., USA
                                  Expairy date: December 2005

Tetracycline   Hovid, Malaysia
                                  Expairy date: June 2005

Tetracycline 3% ointment   SPIC, China
                                                      Expairy date: April 2008

2.1.3. Culture media:

Blood   Police Hospital Patient No. 918

Blood agar base   Mast group Ltd., UK

DNase media   Plasmatec Lab Ltd., UK

K.I.A. media   Mast group Ltd., UK

Koser citrate medium   Oxoid Ltd.

Mac Conkey agar   Scharlu, Spain

Mannitol Salt agar   Oxoid Ltd.

Nutrient agar   Scharlu, Spain

Nutrient broth   Scharlu, Spain

Sabouraud Dextrose agar   Plasmatec Lab. Ltd., UK

Urea agar base   Oxoid Ltd.

2.1.4. Equipment and Instruments:

Autoclave   Baird & Tatlock Ltd.

Balance   A & D Company Ltd.

Centrifuge   Braun, Centrifuge PLC series

Colony counter   Anderman colony counter, Engl.

Glass ware   Griffin & George Ltd.

Hot air oven   Nuve FN 500, Turkey

Incubator   Heraeus

Microscope	Olympus CH-2, Japan
Rota-vap	Buchi, Switzerland
Soxhlet	Grant Instruments Ltd.
Water bath	Fisher scientific, USA

2.1.5. Test micro-organisms:

2.1.5.1. Bacterial micro-organisms:

Bacillus subtilis	NCTC 8236
Staphylococcus aureus	ATCC 25923
Escherichia coli	ATCC 25922
Proteus vulgaris	ATCC 6380
Pseudomonas aeruginosa	ATCC 27853

The standard organisms were obtained from the National Collection of Type Culture (NCTC), Colindale, England and the American Type Culture Collection (ATCC), Rockville, Maryland, USA.

2.1.5.2. Fungal micro-organisms:

| *Aspergillus niger* | ATCC 9763 |
| *Candida albicans* | ATCC 7596 |

2.1.6. Animals:

Swiss Wistar Albino Rats

They were obtained from the National Experimental Animal House (NEAH), Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research (NCR), Ministry of Science and Technology (MOST).

2.2. METHODS:

2.2.1. Plant materials:

The 10 plants used in this study were collected from different parts of Sudan (Refer to Table No.7), by Herbalists in collaboration with the Department of Traditional medicine, from June 2002 to June 2003. They were authenticated by Wai'l Elsadig Abdalla, Medicinal and Aromatic plants Research Institute (MAPRI). Voucher specimens were deposited at the herbarium of the institute.

Data concerning the description of the habitat, the local names, traditional method of preparing the herbal preparations, the way of application and the diseases they were claimed to cure were obtained from the local herbalists and recorded.

50 grams of each plant sample were powdered by grinder and extracted as described in the experimental section and then subjected to antimicrobial activity screening.

Plants screened are listed in table No. (7) with their botanical names, synonyms, families, morphological part used, place of collection, vernacular names and folkloric uses.

2.2.2. Preparation of the 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 (2g) was dissolved or suspended in a mixture containing methanol: petroleum ether (2:1, v/v) to a final volume of 20 ml (con. 100 mg/ml). The methanol residue (2g) was dissolved in methanol 20 ml (con. 100 mg/ml), and kept in a refrigerator until used.

Aqueous extract for each dried ground plant (10g) was prepared by infusion using boiled distilled water. It was allowed to soak for 2 hours, then it was filtered. The residue was then dried and weighed and the yield

percent was obtained. The final volume of the residue was adjusted to 10 ml distilled water and used immediately.

2.2.3. Preparation of 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 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about (10^8-10^9) colony forming units per ml. The suspension was stored in the refrigerator at 4°C until 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 in tubes and (0.02) ml volumes (one drop) of the appropriate dilutions were transferred by Transferpette adjustable volume automatic microtitre pipette onto the surface of dried nutrient agar plates.

The plates were allowed to stand for 2 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 the dilution factor to give the viable count of the stock suspension expressed as the number of colony forming units (C.F.U.)/ per ml of 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.

2.2.3.2. Preparation of fungal suspensions:

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

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.

Three ml of each of the five standardized bacterial stock suspensions (10^8-10^9 C.F.U. /ml) were thoroughly mixed with 300 ml of sterile melted nutrient agar which was maintained at 45°C.

20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates, which were divided to two halves, two cups in each half (10 mm in diameter) were cut using a sterile cork borer (No.4), each one of the 30 halves was designed for one of the 30 extracts.

The agar disks were removed. Alternate cups were filled with 0.1 ml samples of each of the extracts using Transferpette adjustable volume automatic microtitre pipette, and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Two replicates were carried out for each extract against each of the test organisms simultaneously; (positive) controls involving the addition of the respective solvents instead of the extracts were carried out seperately. After incubation the diameters of the resultant growth inhibition zones were measured, mean values were tabulated.

2.2.4.2. Testing for antifungal activity:

The same method as for bacteria was adopted. Instead of nutrient agar, Sabouraud dextrose agar was used. The inoculated medium was incubated at 25°C for two days for the *Candida albicans* and three days for *Aspergillus niger.*

2.2.5. Clinical isolates:

100 clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were collected randomly from the National Health Laboratory, Khartoum Teaching Hospital, Police Teaching Hospital and Khartoum North Teaching Hospital during the period from January to September 2003.

The clinical isolates were obtained from urine, wounds, ear swab, abscesses, sputum and eye swab.

They were purified by streaking on plates containing the appropriate selective and differential culture media. They were identified on the basis of the results of microscopical stain reaction (Gram stain), cultural characteristics and biochemical tests (Cruickshank *et al*, 1975).

2.2.5.1. Media used for identification of clinical isolates:

1- Mannitol salt agar: As a selective medium for the isolation of *Staphylococcus aureus*, which ferment mannitol, giving yellow colour.

2- Mac Conkey agar: As a differential medium for isolation of *Coliforms*.

3- Nutrient agar and Nutrient broth: were used to study the growth and cultural characteristics of the different isolates.

The purified isolates were then subcultured on appropriate slopes prepared from suitable selective media, and then stored at refrigerator until used.

2.2.5.2. Microscopical examination of aerobic bacterial isolates:

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

In principle the bacterial film is fixed and stained with a Triphenylmethane dye such as Crystal violet, in conjunction with Iodine solution, and subsequently treated with an organic solvent such as Alcohol. Bacteria which retain the dye are designated Gram positive and other varieties of bacteria which lose the dye are termed Gram negative. Decolorized organisms are rendered visible by the application of a

counter stain of suitable colour e.g. Safranin red. Shapes and arrangements of the cells were also considered.

2.2.5.3. Biochemical tests:

Species that can not be distinguished by morphology and culture characters may exhibit metabolic differences that can be exploited. It is usual to test the ability of the organisms to produce acidic and gaseous end-products, when presented with individual carbohydrate (glucose, lactose etc…) as the sole carbon source. Other tests determine whether the bacterium produces particular end-product (e.g. Indole) when grown in suitable culture media, and whether it possesses certain enzyme activities, such as Oxidase, Urease etc…

2.2.5.3.1. 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, phenol red as indicator and Durham tube for gas production. After 24 hours incubation, the colour of the inoculated medium 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 (Salle, 1961).

2.2.5.3.2. Methyl-Red test:

It is used to detect the ability of some bacteria to produce significant amounts of acidic substances due to fermentation of glucose. Methyl-Red Voges-Proskauer medium was used. After inoculation, colour changed from yellow (pH 6.2) to red (pH 4.2) with acid production (Cruickshank *et al*, 1975).

2.2.5.3.3. 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 inoculated medium to give a pink colour when alpha-naphthol is added (Cruickshank et al, 1975).

2.2.5.3.4. Citrate utilization test:

It is based upon 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 Koser citrate medium with a 24 hours culture of the tested organism and incubation at 37°C for 2-3 days, and then examination of growth, which changed the colour of the incorporated bromothymol blue indicator from green to blue, due to citrate utilization and production of alkali (Cruickshank *et al*, 1975).

2.2.5.3.5. Indole production test:

It is based on the ability of certain bacteria to oxidize the side chain of the amino acid Tryptophane with the production of indole. Inoculation of the peptone water with the tested organism, incubation for two days and then adding Kovac's reagent changed the colour to red, which indicated the presence of indole (Cruickshank *et al*, 1975).

2.2.5.3.6. Hydrogen Sulphide (H_2S) production test:

It is based upon the ability of some bacteria to produce H_2S from sulfur containing amino acids by reduction. H_2S may be tested by suspending strips of filter paper impregnated with lead acetate above the culture; H_2S was demonstrated by its ability to form black insoluble lead sulfide after incubation of the inoculated peptone water at $37^{o}C$ for 2-3 days (Cruickshank *et al*, 1975).

2.2.5.3.7. Kligler Iron agar (K.I.A.) test:

It is based on the ability of some bacteria to ferment glucose and lactose with the production of gas and H_2S. The tested organism was inoculated and incubated at $37\,^{o}C$ for overnight. Changing the colour of the slope or butt from pink to red indicated fermentation of glucose (alkaline reaction), and changing the colour from pink to yellow indicated fermentation of lactose (acid reaction), (Cheesbrough, 2000).

2.2.5.3.8. Catalase test:

It is based on the presence of the enzyme Catalase in the cells of certain bacteria. The enzyme catalase catalyses the release of oxygen from hydrogen peroxide. The test was carried out by addition of few drops of hydrogen peroxide over a 24 hours nutrient agar culture of the tested

organism, production of gas bubbles indicated a positive Catalase reaction (Salle, 1961).

2.2.5.3.9. Coagulase test:

It is based on the presence of the enzyme coagulase in the cells of some bacteria. 1 in 10 dilution of citrated human plasma in saline was added to few drops of inoculated broth culture with the tested organism, and the mixture was incubated at 37°C and examined for coagulation after 1, 3 and 6 hours. The formation of a clearly visible clot indicated a positive coagulase test (Cruickshank *el al*, 1975).

2.2.5.3.10. Oxidase test:

It is based on the presence of the enzyme Oxidase in the cells of certain bacteria. The oxidase enzymes catalyzes the transport of electrons between electron donors in the bacteria and Tetramethyl-p-phenylenediamine dihydrochloride, a freshly prepared oxidase reagent when added in a solid growth medium, rapidly developed a purple colour at the colonies of oxidase positive organisms (Cruickshank *et al*, 1975; Salle, 1961).

2.2.5.3.11. Urease test:

It is based on the presence of the enzyme Urease in the cells of certain bacteria. Urease enzyme catalyzes the decomposition of urea with the production of ammonia. The test was carried out by growing the tested organism in presence of urea, and testing for ammonia production by means of suitable pH indicator e.g. phenol red. Colour changed from yellow to pink with ammonia production, indicating positive test (Salle, 1961).

2.2.5.3.12. Deoxyribonuclease (DNase) test:

Staphylococcus aureus produces the enzyme Deoxy ribonuclease (DNase). The tested organism was cultured on a medium which contained (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).

2.2.5.4. Testing the susceptibility of clinical isolates 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.

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

The principle of the agar plate dilution method is the inhibition of the growth of the seeded bacteria on the surface of the agar by the incorporated plant extract.

Plates were prepared in series of increasing concentrations of the agent (plant extract), in the following order: 3.125, 6.25, 12.5, 25, 50 and 100 mg/ml.

The bottom of each plate may be marked off into five segments, in the case of standard bacteria, and two segments in the case of fungi. The organism to be tested is grown in broth over-night, and diluted in broth to contain about 10^8 microorganisms per ml.

A loop-ful of the diluted culture is spotted with standard-loop that delivers 0.01 ml onto the surface of each segment and then incubated at 37°C for 18 hours for bacteria and at 25°C for seven days for the fungi.

2.2.6.1. Reading of (MIC) plates:

The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits growth.

Results are reported as the MIC in mg/ml of crude extract.

2.2.7. Antibacterial and Antifungal activity of reference drugs against standard organisms:

Four antibacterial reference drugs (Tetracycline, Ampicillin, Cloxacillin and Gentamicin) and two antifungal reference drugs (Nystatin and Clotrimazole) were prepared in suspensions of four concentrations (40, 20, 10 and 5 μg / ml for all of them except Nystatin which was prepared in 500, 50, 25 and 12.5 μg / ml) using sterile distilled water; to evaluate their antibacterial and antifungal activity against the tested standard

organisms, using the cup plate agar diffusion method previously described in this chapter.

   Removed cups were filled with 0.1 ml samples of each of the four concentrations of the reference drugs using Transferpette adjustable volume automatic microtitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 18 hours.

   Two replicates were carried out for each concentration against each of the test organisms simultaneously. After incubation the diameters of the resultant growth inhibition zones were measured, averages and the mean values were tabulated (Table No. 18).

2.2.8. *In Vivo* Testing of extracts for antimicrobial activity:

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

2.2.8.1. Plant material:

   According to the claimed traditional uses of *Solenostemma argel* as a wound healing agent by Elkamali, 1997 and Shayoub, 2003; and depending on the results of the *in vitro* antimicrobial investigations by Ross *et al,* 1980; Tharib *et al,* 1986; Almagboul 1992 and Elhady *et al,* 1994a; 1994b; the same leaves methanolic extract of *Solenostemma argel* used in the *in vitro* sensitivity tests in this study was employed in the *in vivo* wound healing model.

2.2.8.2. Ointment preparation:

2.2.8.2.1. Petroleum jelly (Vaseline):

   Petroleum jelly B.P. (1988) was used as a hydrocarbon (oleaginous) base to prepare ointments of *Solenostemma argel* extract in 1%, 2 % and 5% concentrations successively.

   The bases were stirred gently by infusion in a water bath till they are homogenously distributed and then cooled.

2.2.8.2.2. Lanolin:

  Lanolin cream was used as an absorptive base to prepare ointments of *Solenostemma argel* extract in 1%, 2 % and 5% concentrations successively.
  The bases were levagated till they take the whole quantity.

2.2.8.2.3. Emulsifying cream (aqueous cream B.P.'1988' -water removable base):

  The bases were prepared from Emulsifying ointment B.P. (1988) in 1%, 2 % and 5% concentrations of *Solenostemma argel* extract, successively.

  The bases were stirred gently by infusion in a water bath till they are homogenously distributed and then cooled.

2.2.8.2.4. Polyethylene Glycol (PEG):

  Polyethylene glycol was used as a water soluble base to prepare ointments of *Solenostemma argel* extract in 1%, 2 % and 5% concentrations successively.

  PEG used: (1: 1) mixture of 400: 4000 PEGs, the mixtures were stirred gently by infusion in a water bath till they are homogenously distributed and then cooled with continuous stirring.

2.2.8.3. 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 Plants Research Institute (MAPRI), National Centre for Research (NCR), Ministry of Science and Technology (MOST), Sudan.

  Rats were housed individually in a ventilated Animal house before and after surgery. They had access to standard diet which has been prepared in (NEAH) supplemented with water *ad libitum* (as much as one likes).

  The holding room was illuminated with 12 hs. Light/ dark cycles. Room temperature was between 30-35°C with 45% to 55% humidity.

2.2.8.4. Tested Microorganisms:

The standard *Staphylococcus aureus* (ATCC 25923) was used.

2.2.8.5. Testing the *In vitro* activity of Ointments:

Measurement of the rates of diffusion of medicament from different bases into an agar gel has been widely employed. For example, the release of an antibacterial agent from ointments may be assessed by measuring the zones of inhibition of bacterial growth around circular cups (Shayoub, 1985).

2.2.8.5.1. The agar-cup plate diffusion method (long period method):

This method was first used by Fuiton and Pablo (1974). It was adopted with some minor modifications, to test the *in vitro* activity of ointments:

240 ml aliquots of sterile melted Nutrient agar was prepared, seeded with 2.4 ml of standardized *Staphylococcus aureus* suspension (10^8-10^9 C.F.U. /ml) at 45°C. The suspension was thoroughly mixed, and poured into 12 Petri-dishes (each 20 ml). Plates were let to solidify in the up-right position.

Using cork borer No. (4); four cups were made in every plate, the agar disks were removed.

Three different concentrations of the four ointments were tested in one of the Petri-dishes.

In every plate, 0.1 g of each preparation was put in each of the four cups, and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37°C for 18 hours.

Additional two plates were prepared using the same method as for the 12 plates (with seeded *Staphylococcus aureus*), four cups were made in every plate, and every one of the bases (without the plant extract) was tested in two cups as a control.

2.2.8.6. First *in vivo* trial: Wound healing activity of *Solenostemma argel* (non-infected rats):

Full thickness wounds were made in the skin of the test 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 the 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 the level of loose subcutaneous tissue, using sterile forceps and a scalpel blade.

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

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

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

Group 1 (wound only):

Untreated control group, wounds were left without treatment.

Group 2 (wound + Tetracycline ointment 3%):

Wounds of these animals were treated topically with Tetracycline ointment 3% every 12 hours as a standard healing agent starting from the first day.

Group 3 (wound + MeOH extract of *S.argel* 2% in PEG):

Wounded animals were treated topically with Polyethylene glycol (water washable base "PEG") ointment which contained 2% of the *Solenostemma argel* leaves methanolic extract every 12 hours starting from the first day.

2.2.8.7. Second *in vivo* trial: Wound healing activity of *Solenostemma argel* (infected rats):

The same steps were done as in the first experiment, but this time with the introduction of artificial wound infection using standard bacteria.

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

Also animals were divided into three groups, each containing five animals:

Group 1 (wound + infection):

Infected control group, wounds were artificially infected with standardized *Staphylococcus aureus* suspension (10^8-10^9 C.F.U. /ml), using Transferpette adjustable volume automatic microtitre pipette to spread 0.1 ml of the suspension in every wound.

Group 2 (wound + Infection + Tetracycline ointment 3%):

Wounds of these animals were artificially infected using the same method as used in Group 1, and treated topically with Tetracycline ointment 3% every 12 hours as a standard healing agent starting from the first day.

Group 3 (wound + Infection + MeOH extract of *S.argel* 2% in PEG):

Wounded animals were artificially infected using the same method as used in Group 1, and treated topically with Polyethylene glycol containing 2% of the *Solenostemma argel* leaves methanolic extract every 12 hours starting from the first day.

The animals were subsequently returned to cages and transported to the holding room.

Beddings in the cages were changed daily and cages were kept clean to avoid contamination.

Application of standard healing agent and the prepared ointment was done twice a day.

All ethical issues were considered in surgery procedure and during the treating period.

2.2.8.8. Evaluation method of the 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 measurement in all calculations.

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

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

2.2.9. Statistical analysis:

Statistical package for social sciences programme (SPSS) version 10 was used.

CHAPTER THREE

RESULTS
&
DISCUSSION

CHAPTER THREE

RESULTS & DISCUSSION

3.1. IDENTIFICATION OF CLINICAL ISOLATES:

In the present work, 100 clinical isolates of *Staphylococcus aureus Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were collected randomly from the National health laboratory, Khartoum teaching hospital, Police teaching hospital and Khartoum North teaching hospital during the period from January to September 2003.

The clinical isolates were obtained from urine, wounds, ear swab, abscesses, sputum and eye swab.

The clinical isolates were identified as previously described. The distribution of these isolates was presented in table No. (2).

3.1.1. Identification of *Escherichia coli*:

3.1.1.1. Cultural characteristics:

On Mac Conkey's agar medium, red colonies were observed as a result of lactose fermentation (Fig. No.3).

3.1.1.2. Microscopical examination:

With Gram staining technique, Gram negative rods were seen.

3.1.1.3. Biochemical reactions:

All isolates fermented lactose, mannitol and glucose with production of acid and gas. Most 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, utilized citrate or gave positive oxidase.

All isolates did not change the yellow colour of K.I.A. both in slope 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 No.2).

3.1.2. Identification of *Proteus vulgaris*:

3.1.2.1. Cultural characteristics:

On Nutrient agar, fishy smell and swarming appearance was clear. On Mac Conkey's agar medium, pale coloured colonies were observed as a sign of non-lactose fermentation (Fig. No.3).

3.1.2.2. Microscopical examination:

With Gram staining technique, Gram negative rods were seen.

3.1.2.3. Biochemical reactions:

All isolates were non-lactose fermenters. Most of them were non-mannitol fermenters.

Most of them did not ferment sucrose with acid production and some formed gas. Most of them fermented glucose, were Methyl Red positive and all were Voges Proskauer 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$_2$S producers and gas non-producers.

All these led to identifying these clinical isolates as *Proteus vulgaris* (Table No.3).

3.1.3. Identification of *Pseudomonas aeruginosa*:

3.1.3.1. Cultural characteristics:

On Nutrient agar, most of the isolates produced blue-green pigments which diffused in the surrounding medium (Fig. No. 4).

3.1.3.2. Microscopical examination:

With Gram staining technique, Gram negative rods were seen.

3.1.3.3. Biochemical reactions:

All isolates were non-lactose fermenters, and most of them fermented glucose and did not ferment sucrose.

Most of them were Methyl Red negative and all were Voges Proskauer negative. Most were oxidase positive, indole negative, did not utilize citrate and did not produce urase.

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 No.4).

3.1.4. Identification of *Staphylococcus aureus:*

3.1.4.1. Cultural characteristics:

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

3.1.4.2. Microscopical examination:

With Gram's staining technique, Gram positive cocci arranged in grape like clusters were seen (Fig. No.1).

3.1.4.3. Biochemical reactions:

Most of the isolates fermented lactose with production of acid and did not form gas. All of them fermented sucrose and mannitol with acid production. Most of them fermented glucose with acid production.

All of them were catalase positive (Fig. No.2), coagulase positive, most were DNase positive.

All these led to identifying these clinical isolates as *Staphylococcus aureus* (Table No.5).
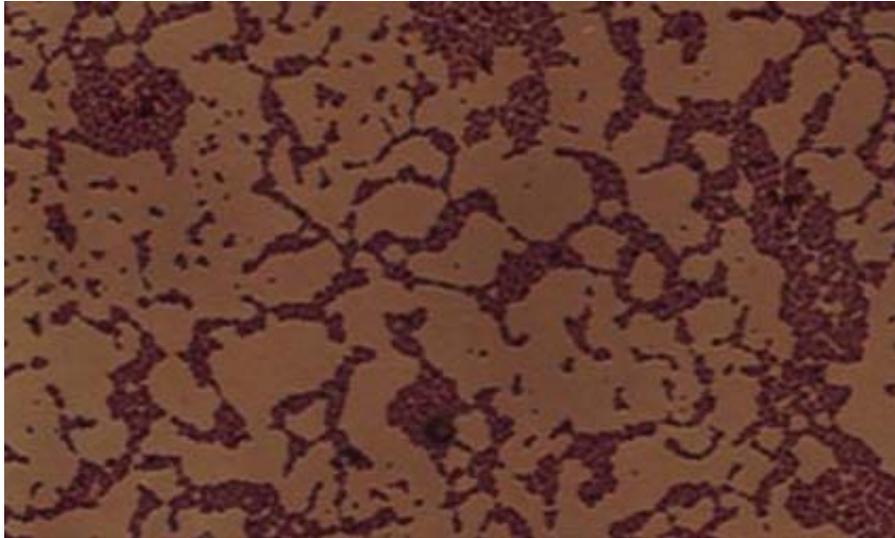
Fig. No. (1)
Gram stain film of *Staphylococcus aureus* arranged in grape like clusters
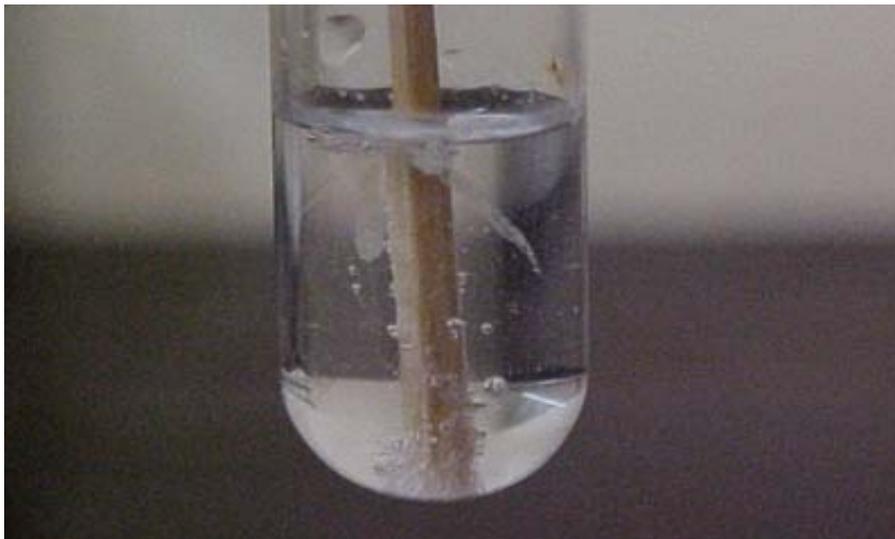
Fig. No. (2)
Positive Catalase test of *Staphylococcus aureus*

Fig. No. (3)
Mac Conkey agar: Left plate exhibits Non-lactose fermenting organism (*Proteus vulgaris*), Right plate exhibits Lactose fermenting organism (*Escherichia coli*)

Fig. No. (4)
Biochemical differentiation of *Pseudomonas aeruginosa*, (from left): Indole (-) test, Citrate control, Citrate (+) test, Urease (+) test, KIA test: red slope & butt, with H₂S and no Gas

Table No. (2): Biochemical tests for the identification of *Escherichia coli* isolates

No.	Suc.	Lact.	Mann.	Gluc.	M.R.	V.P.	Citr.	Ind.	Oxid.	Ur.	K.I.A.				Source
											Slope	Butt	H$_2$S	Gas	
1	+	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
2	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
3	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
4	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
5	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
6	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
7	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
8	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
9	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
10	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
11	+	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
12	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
13	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
14	-	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
15	+	+	+	+	+	-	-	+	-	-	Y	Y	-	+	Urine
SD	85%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	

Suc.= sucrose, Lact.= lactose, Mann.= mannitol, Gluc.= glucose, M.R.= methyl red, V.P.= voges proskauer, Citr.= citrate, Ind.= indole, Oxid.= oxidase, Ur.= urease, Y= yellow; (+): positive test, (-): negative test, S.D. = Significance Degree

Table No. (3): Biochemical tests for the identification of *Proteus vulgaris* isolates

No.	Suc.	Lact.	Mann.	Gluc.	M.R.	V.P.	Citr.	Ind.	Oxid.	Ur.	K.I.A.				Source
											Slope	Butt	H$_2$S	Gas	
1	+	-	-	+	+	-	+	-	-	+	R	Y	+	-	Ear sw.
2	+	-	-	+	+	-	+	-	-	+	R	Y	+	-	Ear sw.
3	-	-	-	+	+	-	-	-	-	+	R	Y	+	-	Ear sw.
4	-	-	-	+	-	-	+	-	-	-	R	Y	+	-	Ear sw.
5	+	-	-	+	+	-	-	-	-	+	R	Y	+	+	Ear sw.
6	-	-	-	+	+	-	+	-	-	+	R	Y	+	-	Ear sw.
7	-	-	-	+	-	-	+	-	-	-	R	Y	+	-	Urine
8	+	-	-	+	+	-	-	-	-	+	R	Y	+	+	Urine
9	-	-	-	+	+	-	+	-	-	+	R	Y	+	-	Urine
10	-	-	-	+	+	-	+	-	-	+	R	Y	+	-	Urine
11	+	-	-	+	-	-	+	-	-	-	R	Y	+	-	Urine
12	-	-	-	+	+	-	+	-	-	+	R	Y	+	-	Urine
13	-	-	-	+	+	-	+	-	-	+	R	Y	+	-	Urine
14	-	-	-	+	+	-	-	-	-	+	R	Y	+	+	Urine
15	+	-	-	+	+	-	+	-	-	+	R	Y	+	-	Urine
16	-	-	-	-	-	-	-	+	-	-	R	Y	-	-	Wound
17	+	-	+	+	+	-	+	-	-	+	R	Y	+	-	Wound
18	-	-	-	-	-	-	+	+	-	+	R	Y	-	+	Wound
SD	61%	100%	94%	88%	72%	100%	72%	88%	100%	77%	100%	100%	88%	77%	

Suc.= sucrose, Lact.= lactose, Mann.= mannitol, Gluc.= glucose, M.R.= methyl red, V.P.= voges proskauer, Citr.= citrate, Ind.= indole, Oxid.= oxidase, Ur.= urease, R= red, Y= yellow; (+): positive test, (-): negative test, S.D. = Significance Degree

Table No. (4): Biochemical tests for the identification of *Pseudomonas aeruginosa* isolates

No.	Suc.	Lact.	Mann.	Gluc.	M.R.	V.P.	Citr.	Ind.	Oxid.	Ur.	K.I.A.				Source
											Slope	Butt	H$_2$S	Gas	
1	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Ear sw.
2	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Ear sw.
3	-	-	-	+	-	-	-	-	-	+	R	R	-	-	Ear sw.
4	-	-	-	+	-	-	+	-	+	-	R	R	-	-	Ear sw.
5	-	-	-	+	-	-	-	-	-	+	R	R	-	-	Ear sw.
6	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Ear sw.
7	-	-	-	-	+	-	-	-	+	-	R	R	+	-	Ear sw.
8	-	-	-	-	-	-	+	-	-	-	R	R	-	-	Ear sw.
9	-	-	-	-	-	-	+	-	+	-	R	R	-	-	Ear sw.
10	-	-	-	-	-	-	+	+	+	+	R	R	+	+	Ear sw.
11	-	-	-	+	-	-	+	-	+	-	R	R	-	-	Urine
12	-	-	-	+	-	-	-	-	-	+	R	R	-	-	Urine
13	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Urine
14	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Urine
15	-	-	-	+	-	-	+	-	+	-	R	R	-	-	Urine

To be continued…

Cont. of Table No. (4): Biochemical tests for the identification of *Pseudomonas aeruginosa* isolates

No.	Suc.	Lact.	Mann.	Gluc.	M.R.	V.P.	Citr.	Ind.	Oxid.	Ur.	K.I.A.				Source
											Slope	Butt	H$_2$S	Gas	
16	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Urine
17	-	-	-	+	-	-	+	-	+	+	R	R	-	-	Urine
18	-	-	-	+	-	-	+	-	-	+	R	R	-	-	Urine
19	-	-	-	+	-	-	-	-	+	+	R	R	-	-	Urine
20	-	-	-	+	+	-	+	-	-	-	R	R	+	+	Urine
21	-	-	-	-	-	-	+	-	+	-	R	R	-	-	Wound
22	-	-	-	-	-	-	+	+	+	-	R	Y	-	+	Wound
23	-	-	-	-	-	-	+	-	+	+	R	R	-	-	Wound
24	+	-	-	+	+	-	+	-	-	+	R	R	+	-	Wound
25	-	-	-	-	-	-	+	-	+	-	R	R	-	-	Wound
26	-	-	-	+	+	-	+	+	+	+	R	Y	+	+	Wound
27	-	-	-	+	+	-	+	-	+	-	R	Y	+	+	Wound
28	-	-	-	-	+	-	+	+	+	-	R	Y	+	+	Wound
29	-	-	-	+	+	-	+	+	+	+	R	Y	-	+	Wound
30	+	-	-	+	+	-	+	+	+	+	R	Y	+	+	Wound

To be continued…

Cont. of Table No. (4): Biochemical tests for the identification of *Pseudomonas aeruginosa* isolates

No.	Suc.	Lact.	Mann.	Gluc.	M.R.	V.P.	Citr.	Ind.	Oxid.	Ur.	K.I.A.				Source
											Slope	Butt	H$_2$S	Gas	
31	+	-	-	+	+	-	+	+	+	+	R	R	+	+	Wound
32	-	-	-	-	-	-	+	-	+	-	R	R	-	-	Wound
33	-	-	-	+	+	-	+	-	-	+	R	R	+	-	Wound
34	+	-	-	+	+	-	-	+	-	+	R	R	+	+	Wound
35	-	-	-	+	+	-	+	+	-	+	R	R	+	+	Wound
36	+	-	-	+	+	-	+	+	+	+	R	Y	+	-	Wound
37	-	-	-	-	-	-	+	-	+	-	R	R	+	-	Wound
38	-	-	-	+	+	-	+	+	+	-	R	R	+	-	Wound
39	-	-	-	-	-	-	-	+	+	-	R	Y	-	+	Wound
40	-	-	-	-	-	-	+	+	+	-	R	Y	-	-	Wound
SD	87%	100%	100%	67%	65%	100%	82%	67%	75%	57%	100%	77%	62%	70%	

Suc.= sucrose, Lact.= lactose, Mann.= mannitol, Gluc.= glucose, M.R.= methyl red, V.P.= voges proskauer, Citr.= citrate, Ind.= indole, Oxid.= oxidase, Ur.= urease, Red= red, Y= yellow; (+): positive test, (-): negative test, S.D. = Significance Degree

Table No. (5): Biochemical tests for the identification of *Staphylococcus aureus* isolates

No.	Catalase	Coagulase	DNase	Sucrose	Lactose	Mannitol	Glucose	Source
1	+	+	+	+	+	+	+	Abscess
2	+	+	+	+	+	+	+	Abscess
3	+	+	+	+	+	+	+	Abscess
4	+	+	+	+	+	+	+	Eye sw.
5	+	+	+	+	+	+	+	Ear sw.
6	+	+	+	+	+	+	+	Ear sw.
7	+	+	+	+	+	+	+	Ear sw.
8	+	+	-	+	+	+	+	Ear sw.
9	+	+	+	+	+	+	+	Ear sw.
10	+	+	+	+	-	+	+	Ear sw.
11	+	+	+	+	+	+	+	Ear sw.
12	+	+	+	+	+	+	+	Ear sw.
13	+	+	-	+	+	+	+	Ear sw.
14	+	+	+	+	-	+	+	Ear sw.
15	+	+	+	+	+	+	+	Ear sw.

To be continued…

Continuation of Table No. (5): Biochemical tests for the identification of *Staphylococcus aureus* isolates

No.	Catalase	Coagulase	DNAse	Sucrose	Lactose	Mannitol	Glucose	Source
16	+	+	+	+	+	+	+	Sputum
17	+	+	+	+	+	+	-	Sputum
18	+	+	+	+	+	+	+	Wound
19	+	+	+	+	+	+	+	Wound
20	+	+	+	+	+	+	+	Wound
21	+	+	+	+	+	+	+	Wound
22	+	+	+	+	+	+	+	Wound
23	+	+	+	+	+	+	+	Wound
24	+	+	+	+	+	+	+	Wound
25	+	+	+	+	+	+	+	Wound
26	+	+	+	+	+	+	+	Wound
27	+	+	+	+	+	+	+	Wound
SD	100%	100%	92%	100%	92%	100%	96%	

(+): positive test, (-): negative test, S.D. = Significance Degree

Table No. (6)

Result of identification of clinical isolates

Bacterial isolates (n = 100)

Type of Bacteria	No. of Isolates	Percentage%
Escherichia coli	15	15%
Proteus vulgaris	18	18%
Pseudomonas aeruginosa	40	40%
Staphylococcus aureus	27	27%

3.2. Screening for antibacterial activity of some Sudanese Medicinal plants:

In the preliminary screening for antibacterial activity of 10 Sudanese medicinal plants, belonging to 10 families, the total number of extracts examined against the five tested organisms was 30; of these extracts, 26 (86.6%) exhibited inhibitory activity against one or more of the five tested bacteria. The other four extracts (13.3%) were devoid of any activity (Table No. 7).

Out of the 26 extracts showing antibacterial inhibitory effects, 8 (30.8%) were inhibitory to the five tested organisms.

Three extracts (11.5%) were active against only one of the five tested bacteria. The number of extracts exhibiting inhibitory effects against two organisms amounted to 5 (19.3%). 4 (15.4%) extracts were active against three organisms and 6 (23%) extracts were active against four organisms.

Staphylococcus aureus was the most sensitive organism (being inhibited by 22 extracts '84.6 %'), while *Proteus vulgaris* showed the lowest susceptibility (being inhibited by 13 extracts '50 %').

19 extracts (73%) exhibited inhibitory effects against *Bacillus subtilis,* whereas 21 (80%) extracts showed inhibitory effects against *Pseudomonas aeruginosa.*

Only 14 extracts (53.8%) gave inhibitory effects against *Escherichia coli.*

16 (61.5%) extracts exhibited inhibitory effects against both Gram positive organisms (*Bacillus subtilis* and *Staphylococcus aureus*), whereas only 10 (38.5%) extracts inhibited the three Gram negative organisms (*Escherichia coli, Proteus vulgaris* and *Pseudomonas aeruginosa*), 6 (23%) extracts inhibited two of the three Gram negative bacteria.

This proves that the Gram negative bacteria were less susceptible to the extracts than the Gram positive organisms.

All chloroformic extracts exhibited inhibitory effects against one or more of the tested organisms.

Only 1 (10%) extract was active against the five tested organisms. 2 (20%) extracts exhibited inhibitory effects against four of the tested organisms. 1 (10%) extract exhibited inhibitory effects against three of the tested organisms. 4 (40%) extracts were inhibitory to two of the bacteria tested.

All methanolic extracts exhibited inhibitory effects against one or more of the tested organisms. 4 (40%) extracts were inhibitory to the five tested organisms. 4 (40%) extracts exhibited effects against four tested organisms. 1 (10%) extract was active against three tested organisms. None of the extracts showed activity against two of the tested organisms.

Out of the 10 aqueous extracts examined for antibacterial activity, 6 (60%) extracts were inhibitory to one or more of the tested organisms. 2 (20%) exhibited activity against the 5 tested organisms. None of the extracts showed activity against 4 of the tested bacteria. 2 (20%) extracts exhibited activity against 3 of the tested bacteria. Antibacterial activity against 2 organisms was shown by 1(10%) extract, while 1 (10%) extract inhibited only 1 organism.

4 (40%) extracts were devoid of any activity against the five tested organisms.

It is evident that the methanolic extracts exhibited the highest level of inhibitory effects against the bacteria tested, this could be due to the presence of polar compounds. 4 methanolic extracts (40%) exhibited inhibitory effects against the five tested bacteria.

The chloroformic extracts showed the lowest level of inhibition against the bacteria tested. Only 1 (10%) extract exhibited inhibitory effect against the five bacteria.

The aqueous extracts showed an intermediate level of inhibition against the tested bacteria, with 2 (20%) extracts exhibiting effects against the five bacteria tested.

3.3. Interpretation of results:

The means of the diameters of the growth inhibition zones obtained in the experiment have been shown in table No. (7), and the results were interpreted in terms of the commonly used terms: sensitive, intermediate and resistant.

On the basis of the results obtained with standard chemotherapeutic agents against the same standard tested organisms (Table No.18), 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 are of intermediate activity, and those resulting in zones below 14 mm are inactive (Cruickshank *et al*, 1975).

3.4. Susceptibility of standard bacteria to different plant extracts:

According to the interpretation of results discussed, the extracts were arranged into three groups according to their activity.

13 extracts exhibited activity against *Staphylococcus aureus,* the same number of extracts showed activity against *Bacillus subtilis*, seven extracts revealed high activity against *Escherichia coli*, whereas only six extracts exhibited high activity against both *Proteus vulgaris* and *Pseudomonas aeruginosa* (Table No. 8).

The percentage of inactive extracts was higher in the case of *Pseudomonas aeruginosa* (73.3%) and both of *Escherichia coli* and *Proteus vulgaris* (70%), when compared to those inactive against *Bacillus subtilis* (43.3%) and *Staphylococcus aureus* (40%), (Table No. 8).

3.4.1. Susceptibility of *Staphylococcus aureus* to different plant extracts:

Regarding the susceptibility of *Staphylococcus aureus* to different extracts, nine methanolic extracts showed high activity, while four aqueous extracts showed high activity and none of the chloroformic extracts showed any activity. The methanolic extracts showed the highest activity against *Staphylococcus aureus* (Table No.9).

3.4.2. Susceptibility of *Bacillus subtilis* to different plant extracts:

Five methanolic extracts were highly active, whereas only four chloroformic and aqueous extracts were highly active against *Bacillus subtilis* (Table No.10).

3.4.3. Susceptibility of *Escherichia coli* to different plant extracts:

None of the chloroformic extracts exhibited any activity against *Escherichia coli*, five methanolic extracts showed high activity, whereas

only two aqueous extracts were highly active against *Escherichia coli* (Table No.11).

3.4.4. Susceptibility of *Proteus vulgaris* to different plant extracts:

  None of the chloroformic extracts showed high activity against *Proteus vulgaris*, four methanolic extracts exhibited high activity against *Proteus vulgaris,* and only two aqueous extracts were highly active against it (Table No. 12).

3.4.5. Susceptibility of *Pseudomonas aeruginosa* to different plant extracts:

  None of the chloroformic extracts showed any activity against *Pseudomonas aeruginosa,* three of the methanolic and three of the aqueous extracts showed high activity against *Pseudomonas aeruginosa* (Table No. 13).

3.5. Screening for antifungal activity of some Sudanese Medicinal plants:

  In this study work, a total of 30 plant extracts from 10 Sudanese medicinal plants from 10 families were screened for their antifungal activity. They were tested against two fungi *Candida albicans* and *Aspergillus niger* (Table No.7).

  The antifungal activities of these plant extracts were compared with those of Nystatin and Clotrimazole as reference antifungal agents (Table No.18).

  Out of the 30 extracts screened, 16 (53.3%) exhibited inhibitory activity against one of the two tested fungi. The other 14 (46.6%) were devoid of any activity against the two tested organisms.

  6 extracts (20%) exhibited inhibitory effect against the two tested organisms, while 10 (33.3%) showed inhibitory effect against only one of the tested organisms. *Candida albicans* was the most sensitive organism (20% of the extracts were active), while *Aspergillus niger* showed the lowest susceptibility (76.6% of extracts were inactive), (Table No.14).

  Out of the 10 chloroformic extracts, 7 (70%) were active against one of the tested organisms.

3 extracts (30%) exhibited inhibitory effect against the two tested organisms. 4 extracts (40%) were active against one organism, while three extracts (30%) were devoid of any activities against the two tested fungi.

7 (70%) of the 10 methanolic extracts exhibited inhibitory effect against one of the two fungi.

Out of the 10 aqueous extracts examined for antifungal activity, only 2 (20%) were active against one of the tested organisms. None of the extracts exhibited inhibition against the two tested organisms. Only 2 (20%) of the extracts were active against one organism, while 8 (80%) were devoid of any activity against the two tested fungi.

It is evident that the chloroformic and methanolic extracts both showed the highest level of antifungal activity. (30%) of the total number of chloroform and methanol extracts examined were inhibitory to the two tested fungi.

The lowest level of antifungal activity was shown by the aqueous extracts, of which none exhibited inhibitory effect against the two fungi.

3.6. Interpretation of results:

According to the interpretation of the results in terms of sensitive, moderate and inactive it had been found that the active extracts against *Candida albicans* were 6 (20%) and those of intermediate activity were 8 (26.6%), and 16 extracts (23.3%) were inactive (Table No.14).

Active extracts against *Aspergillus niger* were only 3 (10%), extracts of intermediate activity were 4 (13.3%) and it exhibited the highest resistance, where 23 (76.6%) extracts were inactive (Table No.15).

3.7. Susceptibility of standard fungi to different plant extracts:

3.7.1. Susceptibility of *Candida albicans* to different plant extracts:

The most active extracts were the methanolic, three of which possessed high activity against *Candida albicans*. Two chloroformic extracts possessed high activity against *Candida albicans,* and only one aqueous extract showed high activity. (90%) of the aqueous extracts were inactive against *Candida albicans,* while only (30%) of both chloroform and

methanol extracts showed activity against *Candida albicans* (Table No.15).

3.7.2. Susceptibility of *Aspergillus niger* to different plant extracts:

Two chloroformic extracts exhibited activity against *Aspergillus niger*, while only one aqueous extract showed antifungal activity against *Aspergillus niger*. None of the methanolic extracts were active against *Aspergillus niger*. (90%) of the aqueous extract were inactive against *Aspergillus niger*, while (70%) of the methanolic and chloroformic extracts were inactive against *Aspergillus niger* (Table No.16).

Table No. (7): Preliminary screening for Antibacterial & Antifungal Activity of some Sudanese medicinal plants

Plant No.	Family/Botanical name/ Synonyms/ Vernacular name	Place of collection	Folkloric Use (local)	Part used (extracted)	Solvent system	Yeild %	Test organism used*/ MDIZ mm**						
							B.s.	S.a.	E.c.	Pr.v.	Ps.a.	A.nig.	C.alb.
1	**Cruciferae** *Raphanus sativus* Radish Vern. Figl	Khartoum state	For acne	Seeds	CHCl$_3$ MeOH H$_2$O	14.97 6.22 3.9	- 12 26.5	12 30 25	12 12 20	- 13 20	- 13 20	17 14 -	- 14.5 20
2	**Tamaricaeae** *Tamarix aphylla* Tamarisk Vern. Tarfa	River Nile banks	For skin diseases	Leaves & Young branches	CHCl$_3$ MeOH H$_2$O	2.26 11.28 2.1	20 27 20	- 25 20	- 15 -	- 20 -	12 20 20	- 15 -	- 21 -
3	**Asclepiadaceae** *Solenostemma argel* Vern. Argel	Nothern state	For colic &bacterial infections	Leaves	CHCl$_3$ MeOH H$_2$O	6.33 15.1 1.9	- 15 -	- 28 15	- - -	- 22.5 -	11 12 -	- - -	24.5 - -
4	**Mimosaceae** *Mimosa pigra L.* Thorn blood Vern.Shagarat Alfas	River Nile banks	For Ecthema	Leaves	CHCl$_3$ MeOH H$_2$O	5.1 14.12 1.7	20 30 -	- 18 -	- 20 -	- 20 -	12 22 -	- - -	16 18.5 -
5	**Combretaceae** *Guiera senegalensis* Vern. Ghubeish	Nuba mountains	Antipyretic, Antidiabetic	Leaves	CHCl$_3$ MeOH H$_2$O	3.82 18.41 6.2	- 25 21	13 20 15	12 25 20	12 20 20	12 25 22	12.5 15 -	17 22.5 -

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Con. of Table No.(7):Preliminary screening for Antibacterial & Antifungal Activity of some Sudanese medicinal plants

Plant No.	Family/Botanical name/ Synonyms/ Vernacular name	Place of collection	Folkloric Use (local)	Part used (extracted)	Solvent system	Yeild %	Test organism used*/ MDIZ mm**						
							B.s.	S.a.	E.c.	Pr.v.	Ps.a.	A.nig.	C.alb.
6	**Menispermaceae** *Tinospora bakis* *Cocculus bakis* Vern. Irg Alhagar	Nuba mountains	For headaches	Root	CHCl₃ MeOH H₂O	1.28 6.67 2.6	12 19 -	- 25 -	- - -	- 15 -	- 11 -	- - -	14.5 15.5 -
7	**Rubiaceae** *Xeromphis nilotica* Vern. Shagarat Elmarfien	Nuba mountains	Juandice, Rabies	Branches	CHCl₃ MeOH H₂O	0.44 4.33 1.8	20 - -	12.5 20 -	12 17 -	- 15 -	11 11 -	21 - -	20 15 -
8	**Polygalaceae** *Securidaca longepedunculata* Vern. Irg Alali	Blue Nile state	Headache, Stomach pain	Root	CHCl₃ MeOH H₂O	1.6 19.08 3.6	15 - -	12 25 -	- 20 -	- - -	11 11 -	40 - -	16 - -
9	**Bignoniaceae** *Kigelia Africana* *Bignonia Africana* Vern. Umm mashatour	Khartoum state	Breast cancer, Leshmania	Fruits	CHCl₃ MeOH H₂O	0.65 20.24 3.5	25 15 23	15 29.5 20	12 22 -	15 13 -	13 15 -	- - -	- 14 -
10	**Capparidaceae** *Courbonia virgata* Vern. Kordala	Nuba mountains	Syphlis, Rematoids	Roots	CHCl₃ MeOH H₂O	1.08 14.31 5.3	15 25 -	17 31.5 25	- 34 -	- - 13	- 12 15	- - 22.5	- - -

* S.a. = *Staphylococcus aureus*, B.s. = *Bacillus subtilis,* E.c. = *Escherichia coli*, Pr.v. = *Proteus vulgaris*, Ps.a. = *Pseudomonas aeruginosa*, A.nig. = *Aspergillus niger*, C.alb. = *Candida albicans*. **: Mean diameter of growth inhibition zones in mm. Average of 2 replicates. (-): no inhibition. Cup diameter = 10 mm.

Table No. (8)

Susceptibility of Standard Organisms to different plant extracts*

organism	No. of extracts		
	active**	moderate***	inactive****
Staphylococcus aureus	13	5	12
Bacillus subtilis	13	4	13
Escherichia coli	7	2	21
Proteus vulgaris	6	3	21
Pseudomonas aeruginosa	6	2	22

*   No. of extracts = 30
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (9)

Susceptibility of *Staphylococcus aureus* to different plant extracts*

Solvent (extract)	No. of extracts		
	active**	moderate ***	inactive ****
$CHCl_3$	0	2	8
MeOH	9	1	0
H_2O	4	2	4

*   No. of each extract = 10
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (10)

Susceptibility of *Bacillus subtilis* to different plant extracts*

Solvent (extract)	No. of extracts		
	active**	moderate***	inactive ****
CHCl$_3$	4	2	4
MeOH	5	2	3
H$_2$O	4	0	6

*   No. of each extract = 10
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (11)

Susceptibility of *Escherichia coli* to different plant extracts*

Solvent (extract)	No. of extracts		
	active **	moderate***	inactive ****
CHCl$_3$	0	0	10
MeOH	5	2	3
H$_2$O	2	0	8

*   No. of each extract = 10
**   Active $\equiv$ M.D.I.Z. > 18 mm
***   Moderately active $\equiv$ M.D.I.Z. = 14-18 mm
**** Inactive $\equiv$ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (12)

Susceptibility of *Proteus vulgaris* to different plant extracts*

Solvent (extract)	No. of extracts		
	active **	moderate ***	inactive ****
CHCl$_3$	0	1	9
MeOH	4	2	4
H$_2$O	2	0	8

*   No. of each extract = 10
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (13)

Susceptibility of *Pseudomonas aeruginosa* to different plant extracts*

Solvent (extract)	No. of extracts		
	active **	moderate ***	inactive ****
$CHCl_3$	0	0	10
MeOH	3	1	6
H_2O	3	1	6

* No. of each extract = 10
** Active ≡ M.D.I.Z. > 18 mm
*** Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (14)

Susceptibility of Standard Fungi to different plant extracts*

organism	No. of extracts		
	active **	moderate ***	inactive ****
Aspergillus niger	3	4	23
Candida albicans	6	8	16

*   No. of extracts = 30
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (15)

Susceptibility of *Candida albicans* to different plant extracts*

Solvent	No. of extracts		
(extract)	active **	moderate ***	inactive ****
CHCl$_3$	2	5	3
MeOH	3	4	3
H$_2$O	1	0	9

*   No. of each extract = 10
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

Table No. (16)

Susceptibility of *Aspergillus niger* to different plant extracts*

Solvent (extract)	No. of extracts		
	active **	moderate ***	inactive ****
CHCl$_3$	2	1	7
MeOH	0	3	7
H$_2$O	1	0	9

*   No. of each extract = 10
**   Active ≡ M.D.I.Z. > 18 mm
***   Moderately active ≡ M.D.I.Z. = 14-18 mm
**** Inactive ≡ M.D.I.Z. < 14 mm
M.D.I.Z. = Mean diameter inhibition zone

3.8. The Antibacterial and Antifungal activity of the plants:

3.8.1. *Raphanus sativus L.:*

In this study, the chloroformic seeds extract exhibited no activity against *Bacillus subtilis, Proteus vulgaris, Pseudomonas aeruginosa* and *Candida albicans*. The extract showed poor activity against *Staphylococcus aureus* and *Escherichia coli*, but it showed moderate activity against *Aspergillus niger*.

The methanolic extract showed high activity against *S. aureus*, moderate activity against *Aspergillus niger* and poor activity against the rest of the tested organisms.

Regarding the aqueous extract, high activity resulted against the five tested bacteria and *C. albicans*, but no result was given against *A. niger*.

Saxena and Vyas (1986) screened the petroleum ether and ethanol extracts of seeds of 14 Indian plants for their *in vitro* activity against human pathogenic fungi (*Aspergillus fumigatus, Trichophyton mentagrophytes* and *Candida albicans*) and bacteria (*Escherichia coli, Bacillus subtilis* and *Streptococcus faecalis*). Antifungal and antibacterial activity was demonstrated by four plants including *Raphanus sativus*.

In this study the antimicrobial properties of the chloroformic, methanolic and water extracts were studied, whereas Uda *et al.* (1993) concluded that the antimicrobial properties of water soluble products obtained by degradation of 4-methylthio-3-butenyl isothiocyanate, the pungent principle from radish (*Raphanus sativus*), were studied using five Gram negative, five Gram positive bacteria, five yeasts and nine fungi. Two antimicrobial components were found in the water soluble products. The identified compounds exhibited a prominent growth inhibition on the fungi and Gram positive bacteria.

In 1996 Ela *et al.* evaluated the antimicrobial activity of 16 essential oils, from Egyptian plants including *Raphanus sativus*, against *Escherichia coli, Staphylococcus aureus, Aspergillus niger, Candida albicans* and *Chaetomium olivacum*. All the essential oils studied showed potent activities against at least one of the tested organisms.

Gao *et al.* (1997) performed primary studies on the bacteriostasis and fungistasis of lysozyme from *Raphanus sativus*. Growth was inhibited by varying degrees.

The result of the antimicrobial activity studied by Uda *et al* (1993) using the water extract of this plant was similar to the result obtained in this study, and this is suggested to using the same solvent system.

It is worth mentioning that the World Intellectual Property Organization (WIPO) had registered numerous patents for medicinal uses of this plant world-wide, including using the plant as topical treatment for Psoriases (from the seeds extract), anti-irritant and virus proliferation inhibitor.

3.8.2. *Tamarix aphylla*:

In the present work, chloroform extract of the leaves and young branches showed no inhibitory effect against all tested organisms, except *Bacillus subtilis*, which showed high activity; and against *Ps. aeruginosa,* which showed poor activity.

The methanolic extract showed high activity against the five tested bacteria and also the two fungi.

The aqueous extract exhibited high activity against the two Gram positive bacteria tested, and *Ps. aeruginosa*. It showed poor results against *E.coli, Pr. vulgaris* and the two fungi.

Maoz and Neeman (1998) tested the aqueous extracts of 10 plants from Israel including the bark of *Tamarix aphylla*, for their ability to inhibit *T. rubrum, M. canis, Bacillus subtilis, Sarcina lutea* and *Staphylococcus aureus*.

The results obtained in this study against *S. aureus* and *B. subtilis* were contrary to those of Maoz and Neeman (1998) that is suggested because of using different plant parts from different habitats, but there is an agreement about the fungal activity.

3.8.3. *Solenostemma argel* (Del.) Hayne:

In the present work, the chloroformic leaves extract showed no activity against all tested organisms, except for *Ps. aeruginosa* where the result was poor and *C.albicans* which gave very high results.

Regarding the methanolic extract, it showed high activity against *S. aureus* and *Pr. vulgaris,* and moderate results against *B. subtilis* and *Ps. aeruginosa.* No results were obtained against *E.coli, A.niger* and *C.albicans.*

With respect to the aqueous extract, moderate activity was obtained against *S.aureus,* and no activity against the rest four bacteria and two fungi.

Ross *et al*. (1980) selected the alcoholic extracts of 32 plants on the basis of literature references and use in folklore, and studied them for their activity against four pathogenic bacteria and five fungi. Only eight of the plants, including *Peganum harmala* and *Tamarindus indica*, showed high antibacterial activity. Five plants, including *P. Harmala* and *Solenostemma argel,* were markedly antifungal. There was a correlation between the antimicrobial activity and the contents of physiologically active principles.

Tharib *et al.* (1986) screened four compounds isolated from the stems of the desert shrub *Solenostemma argel*, which is used in local medicine in Libya. Only one (from the saponifiable fraction) showed activity against both Gram positive and Gram negative bacteria.

Almagboul (1992) reported *Solenostemma argel* as one of the plants which exhibited antimicrobial activity in her study.

In this study the leaves extract (aerial parts) was studied and Elhady *et al.* (1994a) studied the antimicrobial activity of the aerial parts of *Solenstemma argel*, which were successively extracted with methanol/water in different proportions (4 fractions). Eight bacteria were used: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Protus vulgaris*, *Klebsiella pneumoniae*, *Micrococcus* and *Streptococcus spp.* 14 fungi were tested including: *Aspergillus niger* and *Candida albicans.* The greatest effect was observed for *Streptococcus* species and moderate action against *E. coli, S. aureus*, K. *pneumoniae* and *P. vulgaris*. Fraction No. 1 showed antifungal activity against *A. niger*, while fraction No. 2 showed activity against *C. albicans*. Fration No. 3 showed Activity against both fungi, while fraction No. 4 showed high activity against *A. parasiticus* and *A. candidus*.

Elhady *et al.* (1994b) studied the antimicrobial activity of the aerial parts of *Solenstemma argel*, which were successively extracted with chloroform/methanol in different proportions (4 fractions). Eight bacteria were used: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Protus vulgaris*, *Klebsiella pneumoniae*, *Micrococcus* and *Streptococcus spp.*14 fungi were tested including: *Aspergillus niger* and *Candida albicans.* The greatest effect was observed

against *Streptococcus* species and moderate action against *E. coli, S. aureus*, *K. pneumoniae* and *P. vulgaris*. There was no effect on Micrococcus and *Pseudomonas aeruginosa,* while a weak antifungal activity was observed.

Results obtained in this study are similar to the results obtained by Elhady *et al.* (1994a; 1994b) and that is suggested to the use of the same solvents and plant parts.

A single patent was the result of search in (WIPO) data-bases, which showed that *Solenstemma argel* is used orally and nasally with a mixture of other eight plants for treatment of viral hepatitis diseases. The mixture of oral herbs results in stimulation of regeneration of liver cells, reduction of blood cholesterol, increased bilirubin secretion and excretion, and stimulation of the lymphocytic system. The compositions are non-toxic, although they may have a bad taste and cause some sneezing.

3.8.4. *Mimosa pigra*:

In the present work, chloroform extracts of the leaves showed high activity against *B. subtilis*, poor activity against *Ps. aeruginosa* and no activity against the other three tested bacteria, whereas it showed moderate activity against *C.albicans* and no activity against *A.niger*.

The methanolic extract showed high activity against the five tested bacteria, and *C.albicans;* while it revealed no activity against *A.niger.*

The aqueous extract showed no activity against the five tested bacteria and the two fungi.

In this study the chloroformic, methanolic and aqueous extracts were studied and Rosado *et al.* (2000) studied the methanol and water extracts of six Fabaceae species including *Mimosa pigra*; which are traditionally used in Mayan medicine for the treatment of diarrhea and eye infections, for their *in vitro* antimicrobial activity. *Mimosa pigra* inhibited the growth of *Pseudomonas aeruginosa*, but showed no activity against *E.coli.* It also has antifungal activity.

Ali *et al.* (2001) screened the antimicrobial activity of 11 Mimoaceous plants against various bacteria and fungi and found them to be active.

Results obtained by Rosado *et al.* (2000) against *Pseudomonas aeruginosa* and *candida albicans* are similar to the results obtained in this

study, may be due to the use of the same solvents, but the results by the former against *E.coli* and *Aspergillus niger* were different and that is suggested to the difference in habitat.

3.8.5. *Guiera senegalensis* **J.F. Gmelin:**

In the present study the chloroformic leaves extract showed poor activity against *S. aureus, E.coli, P. vulgaris* and *Ps. aeruginosa.* It showed no activity against *B. subtilis*. Moderate activity was shown against *C.albicans* and poor activity resulted against *A.niger*.

The methanolic extract showed very high activity against the five tested bacteria and the two fungi.

The aqueous extract exhibited very high activity against the five tested bacteria, but no activity was shown towards the two fungi.

Grand (1989) reported the result of a literature survey of the ethnobotanical and pharmacological data concerning 43 species used by the Diola tribe against Fungal and Bacterial infections. 10 of these plants, including *Guiera senegalensis*, showed antimicrobial activity which he associated with the presence of pharmacologically active substances.

In the present study the leaves extract was tested, whereas Bosisio *et al.* (1997) studied the biological activity of *Guiera senegalensis*. Its leaves are used in African traditional medicine for gastrointestinal disorders, coughs and topically for wound healing. The antimicrobial activity of the leaf extracts were examined against Gram positive, Gram negative bacteria, moulds and yeasts. The crude extract possessed a mild antimicrobial effect only on Gram positive bacteria (MIC 0.8-1.5 mg/ml). Results obtained in this study using the leaves extract are greater than the results obtained by Bosisio *et al.*(1997), and that is may be due to using different solvents.

Silva *et al.* (1997) studied the *in vitro* antibacterial activity of eight medicinal plants from Guinea-bissau including: *Guiera senegalensis*, against *Neisseria gonorrhoeae*. These plants are used by Fulani traditional healers to treat several disorders including venereal diseases. All extracts exhibited antibacterial activity against all the tested strains of *Neisseria gonorrhoeae*.

Sanogo *et al.* (1998) evaluated the antibacterial properties of a decoction and methanol extract of 13 plant species used in the traditional medicine

of Mali, to treat fever and respiratory tract diseases; against clinically isolated bacterial strains responsible for respiratory infections. The extracts of *Guiera senegalensis* and other two plants exhibited significant activity against all strains of bacteria tested.

Silva and Gomes (2003) isolated a new compound from the leaves of *Guiera senegalensis*, which exhibited potent antifungal activity against *Cladosporium cucumerinum*.

Results obtained by Sanogo *et al.*(1998) using the methanolic extract against bacteria are similar to the results obtained in this study and that is suggested to the use of the same solvents.

3.8.6. *Tinospora bakis* (A. Rich) Miers:

In this investigation, the chloroform extract of the root gave poor result against *B. subtilis*, and no result against *S. aureus*, *E. coli, Pr. vulgaris* and *Ps. aeruginosa,* while it gave moderate results against *C. albicans* and no result against *A. niger.*

The methanolic extract gave high activity against *S. aureus,* moderate activity against *B. subtilis* and *Pr.vulgaris,* poor results against *Ps. aeruginosa* and no result against *E. coli,* while it showed moderate activity against
C. albicans and no activity against *A. niger.*

The aqueous extract did not show any activity.

No literature was encountered in correspondence of this plant.

3.8.7. *Xeromphis nilotica*:

In this study the chloroformic extract of the branches showed high activity against *B. subtilis*, poor activity against *S. aureus*, *E. coli* and *Ps. aeruginosa;* and no activity against *Pr. vulgaris*, whereas it showed very high activity against the two tested fungi.

The methanolic extract showed high activity against *S. aureus*, moderate activity against *E. coli* and *Pr. vulgaris*, poor activity against *Ps.aeruginosa* and no activity against *B. subtilis.* It showed moderate activity against *C. albicans*, but it had no activity against *A. niger*.
The aqueous extract showed no activity at all against all tested organisms.

In the present work the branches extracts were tested, whereas Almagboul (1992) screened the antibacterial activity of some Sudanese medicinal plants including: *Xeromphis nilotica*. She tested the Chloroformic, Methanolic and Aqueous extracts of the plant against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* The fruit chloroformic and methanolic extracts of *Xeromphis nilotica* were only active against *S. aureus*, but the fruit aqueous extract was active against all tested organisms.

Results obtained from testing the water extract by Almagboul (1992) were contrary to the results obtained in this study and that may be due to using different plant parts, but both results were similar in regard of *S. aureus*.

3.8.8. *Securidaca longepedunculata* (Fresen.), Mus. Senk:

In the present study the chloroformic root extract showed moderate activity against *B. subtilis,* poor activity against *S. aureus* and *Ps. aeruginosa.* No activity against *E. coli* and *Pr. vulgaris* was obtained. While it showed very high activity against *A. niger* and moderate activity against *C. albicans*.

The methanolic extract showed high activity against *S. arueus* and *E. coli,* poor activity against *Ps. aeruginosa*, and no activity was shown against *B. subtilis, P. vulgaris* and the two tested fungi.

The aqueous extract showed no activity against all tested bacteria and fungi.

Almagboul (1992) screened the antibacterial activity of some Sudanese medicinal plants including: *Securidaca longepedunculata*. She tested the Chloroformic, Methanolic and Aqueous extracts of each of these plants against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* All leaves extracts of *Securidaca longepedunculata* were active against the tested organisms.

Omer (2000a) screened 44 Sudanese medicinal plants (including *Securidaca longepedunculata*), for their *in vitro* antimicrobial activity against four bacteria and three fungi. The chloroformic and methanolic bark and root extracts of *Securidaca longepedunculata* gave significant results against all tested microorganisms, while the aqueous extracts of the bark and root were inactive against the tested microorganisms.

The antimicrobial activity studied by Almagboul (1992) and Omer (2000a) were similar, whereas the results of chloroform and methanol extracts obtained in this work is different from them due to using different plant parts. Result of testing the aqueous extract in this work is similar to the results of Omer (2000a).

3.8.9. *Kigelia africana* (Lam.) Benth.:

In the present study the chloroformic fruit extract showed high activity against *B. subtilis*, moderate against *S. aureus* and *Pr. vulgaris*, poor results against *E. coli* and *Ps. aeruginosa*. No result was obtained against the two tested fungi.

The methanolic extract showed high activity against *S. aureus* and *E. coli*, moderate activity against *B. subtilis* and *Ps. aeruginosa*, and poor results against *Pr. vulgaris*. Moderate activity was shown towards *C. albicans* and no activity against *A. niger* was shown.

The aqueous extract exhibited very high activity against *S. aureus* and *B. subtilis* and nothing towards the other three bacteria and two fungi.

Almagboul (1992) screened the antibacterial activity of some Sudanese medicinal plants including: *Kigelia africana*. She tested the Chloroformic, methanolic and aqueous extracts of each of these plants against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The fruit chloroformic and methanolic extracts of *Kigelia Africana* gave good results, while the aqueous extracts were inactive against the tested organisms.

Kwo *et al.* (1996) screened the concentrated bark extracts of three Cameroonian medicinal plants (*Alstonia boonei, Kigelia Africana* and *Morinda lucida*), for their antimicrobial activity using the disk diffusion susceptibility test. Solvents with different polarity were used for the extraction (methylene chloride, ethyl acetate, 95% ethanol and acetonitrile), and the extracts were tested against five human pathogens: *Candida albicans, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis ' Streptococcus faecalis'* and *Pseudomonas aeruginosa*. The patterns of inhibition varied with the plant extract, the solvent used for extraction, and the organism tested. The largest zones of inhibition were observed for ethanol extracts of *kigelia Africana* against *Staphylococcus aureus and Pseudomonas aeruginosa. Staphylococcus aureus* was the most susceptible microorganism. No inhibitory effects

were observed against *C. albicans*. The extent of inhibition of the bacteria was related to the concentration of the plant extract.

Grace *et al*. (2002) who 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 plate assay. All extracts showed significant activity against the tested Gram positive and Gram negative bacteria, supporting the traditional use of the plant in the therapy of bacterial infections.

In 2003 Ijah and Oyebanji determined the chemical composition of *Enantia chlorantha, Kigelia Africana, Bridelia ferruginea, Trema nitems* and *Drypetes gossweileri*, collected from Nigeria, using thin layer chromatography. Alongside this, the activity of the crude aqueous and ethanolic extracts of the medicinal plants species against *E. coli, S. aureus, Ps. aeruginosa, Klebsiella sp.*and *Proteus sp.*, causing urinary tract infection was determined. The extracts of the medicinal plant species inhibited the growth of the bacteria tested.

The results obtained in this study are similar to all the above listed results, but they are contrary to those of Almagboul (1992) as she reported that the fruit aqueous extract as inactive against the tested bacteria, while in this study the fruit aqueous extract was active against the Gram positive bacteria.

Some patents were registered in the (WIPO) in regard of the medicinal uses of *Kigelia Africana* including using it for the treatment of Inflammation, Alzheimer's disease, Parkinson's disease and Arthritis.

3.8.10. *Courbonia virgata* Brongn.:

In the present study the chloroformic root extract showed moderate activity against Gram positive bacteria and no activity against neither the Gram negative bacteria nor the fungi.

The methanolic extract exhibited very high activity against *S. aureus, B. subtilis* and *E. coli,* poor activity was shown against *Ps. aeruginosa* and no activity was shown against *Pr. vulgaris* or the two fungi.

The aqueous extracts revealed high activity against *S. aureus,* moderate activity against *Ps. aeruginosa,* poor activity against *Pr. vulgaris* and no activity against *B. subtilis* and *E. coli*.

It showed high activity against *A.niger* and no activity against *C.albicans.*

No literature was encountered in correspondence of this plant.

3.9. Determination of the Minimum Inhibitory Concentrations (MICs):

The minimum inhibitory concentrations of the most active extracts (i.e. the 10 methanolic extracts of the 10 studied medicinal plants) were determined against the standard organisms (*Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger*).

The results were summarized in (Table No.17) as mg/ml of crude extract.

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

Staphylococcus aureus is the most susceptible organism; Gram positive bacteria are more susceptible than Gram negative.

3.9.1. *Raphanus sativus* MIC:

The MIC against *Staphylococcus aureus* was the lowest. The MICs against the Gram negative bacteria and the two fungi were the highest, i.e. this plant had weak activity.

3.9.2. *Tamarix aphylla* MIC:

The MICs against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the lowest, i.e. the plant extract was active against these organisms, whereas the MIC of the extract against *Escherichia coli* was higher.

The plant extract had no activity against the two fungi in up to 100 mg/ml concentration.

3.9.3. *Solenostemma argel* MIC:

The plant extract's MIC against *Staphylococcus aureus* was very low showing the high activity of the plant extract against that organism. The plant extract had no activity against both Gram negative bacteria and the two tested fungi.

3.9.4. *Mimosa pigra* MIC:

The MICs of this plant extract were very low against the five tested bacteria suggesting that it contains a good antibacterial natural agent, especially against *Staphylococcus aureus*.

The plant extract was inactive against the two tested fungi resulting in the highest MICs.

3.9.5. *Guiera senegalensis* MIC:

Plant extract gave low MICs against the Gram positive and Gram negative bacteria showing the high activity of the plant, especially in the case of *Staphylococcus aureus*.

The plant extract showed high MICs against *A. niger* and *C. albicans*.

The plant extract did not solidify with the media in 100 mg/ml concentration, may be due to the presence of tannins in the plant extract.

3.9.6. *Tinospora bakis* MIC:

The plant extract showed the highest MICs against all tested organisms (greater than 100 mg/ml), i.e. that it is inactive in this concentration.

3.9.7. *Xeromphis nilotica* MIC:

MICs were high against all tested organisms, except in the case of *Staphylococcus aureus* which showed the lowest MIC.

3.9.8. *Securidaca longepedunculata* MIC:

MICs of the plant extract of this plant against all tested organisms were the highest (MICs > 100 mg / ml), i.e. it is not active against the organisms in100 mg/ml concentration.

3.9.9. *Kigelia africana* MIC:

MICs of this plant extract against the Gram positive bacteria were lower than those against the Gram negative bacteria and the two tested fungi.

3.9.10. *Courbonia virgata* MIC:

MICs of this plant methanolic extract against *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* were lower than MICs against *Escherichia coli* and *Pseudomonas aeruginosa*.

MICs of the plant extract were high against the two tested fungi.

3.10. Antibacterial and Antifungal activity of reference drugs against standard organisms:

The antibacterial and antifungal activity of the selected reference drugs against standard organisms was determined using four different concentrations of the reference drugs.

The results were summarized in table No.(18)

3.10.1. Comparing the activity of *Raphanus sativus* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *A. niger* with inhibition action similar to that of 50 μg / ml of Nystatin.

The plant methanolic extract inhibited *B. subtilis* similarly to 5 μg / ml of Ampicillin, and inhibited *S. aureus* similarly to 40 μg / ml of Tetracycline and 10 μg / ml of Gentamicin; and higher than 40 μg / ml of Ampicillin and Cloxacillin. It inhibited *Ps. aeruginosa* similarly to 20 μg / ml of Tetracycline, and inhibited *A. niger* similarly to 25 μg / ml of Nystatin.

The plant aqueous extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline and Ampicillin and higher than 20 μg / ml of Gentamicin.
It inhibited *S. aureus* similarly to 10 μg / ml of Tetracycline, 40 μg / ml of Ampicillin and 20 μg / ml of Cloxacillin. It inhibited *E. coli* higher than 10 μg / ml of Gentamicin; inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline and inhibited *Ps. aeruginosa* higher than 40 μg / ml of Tetracycline and 5 μg / ml of Gentamicin.

3.10.2. Comparing the activity of *Tamarix aphylla* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* higher than 40 μg / ml of Ampicillin and similarly to10 μg / ml of Tetracycline and 10 μg / ml of Gentamicin. It inhibited *Ps. aeruginosa* similarly to 10 μg / ml of Tetracycline.

The plant methanolic extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline and Ampicillin and higher than 20 μg / ml of Gentamicin.

It inhibited *S. aureus* similarly to 40 μg / ml of Ampicillin, 10 μg / ml of Tetracycline and 20 μg / ml of Cloxacillin. It inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline. It inhibited *Ps. aeruginosa* higher than 40 μg / ml of Tetracycline and 5 μg / ml of Gentamicin and inhibited *A. niger* higher than 25 μg / ml of Nystatin.

The plant aqueous extract inhibited *B. subtilis* similarly to the action of the chloroformic extract, and inhibited *S. aureus* similarly to 20 μg / ml of Ampicillin, 10 μg / ml of Cloxacillin and higher than 5 μg / ml of Tetracycline. It inhibited *Ps. aeruginosa* similarly to the action of the methanolic extract.

3.10.3. Comparing the activity of *Solenostemma argel* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *C. albicans* higher than 12.5 μg / ml of Nystatin.

The plant methanolic extract inhibited *B. subtilis* similarly to 40 μg / ml of Ampicillin. It inhibited *S. aureus* higher than 40 μg / ml of Ampicillin and 20 μg / ml of Tetracycline and similarly to 40 μg / ml of Cloxacillin and 5 μg / ml of Gentamicin. It inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline and 5 μg / ml of Gentamicin. It inhibited *Ps. aeruginosa* similarly to 10 μg / ml of Tetracycline.

The plant aqueous extract inhibited *S. aureus* similarly to 5 μg / ml of Ampicillin and Cloxacillin.

3.10.4. Comparing the activity of *Mimosa pigra* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* higher than 40 μg / ml of Ampicillin and similarly to10 μg / ml of Tetracycline and 10 μg / ml of Gentamicin. It inhibited *Ps. aeruginosa* similarly to 10 μg / ml of Tetracycline.

The plant methanolic extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline, Ampicillin and Gentamicin. It inhibited *S. aureus* higher than 5 μg / ml of Tetracycline and Cloxacillin, and similarly to10 μg / ml of Ampicillin. It inhibited *E. coli* higher than 10 μg / ml of Gentamicin, and inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline. It inhibited *Ps. aeruginosa* higher than 40 μg / ml of Tetracycline and similarly to 20 μg / ml of Gentamicin.

3.10.5. Comparing the activity of *Guiera senegalensis* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *Ps. aeruginosa* similarly to10 μg / ml Tetracycline.

The plant methanolic extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline and Ampicillin, and 20 μg / ml of Gentamicin. It inhibited *S. aureus* similarly to 20 μg / ml of Ampicillin, 10 μg / ml of Cloxacillin and higher than 5 μg / ml of Tetracycline. It inhibited *E. coli* higher than 10 μg / ml of Gentamicin and inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline. It inhibited *Ps. aeruginosa* higher than 40 μg / ml of Tetracycline and Gentamicin. It inhibited *A. niger* higher than 25 μg / ml of Nystatin and inhibited *C. albicans* similarly to 12.5 μg / ml of Nystatin.

The plant aqueous extract inhibited *B. subtilis* higher than 40 μg / ml of Ampicillin and10 μg / ml of Gentamicin and similarly to 20 μg / ml of Tetracycline. It inhibited *S. aureus* similarly to 5 μg / ml of Ampicillin and Cloxacillin, It inhibited *E. coli* higher than 10 μg / ml of Gentamicin, and inhibited *Pr. vulgaris* higher than 40 μg / ml of Tetracycline; It inhibited *Ps. aeruginosa* higher than 40 μg / ml of Tetracycline and similarly to 20 μg / ml of Gentamicin.

3.10.6. Comparing the activity of *Tinospora bakis* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* similarly to 5 μg / ml of Ampicillin.

The plant methanolic extract inhibited *B. subtilis* higher than 40 μg / ml of Ampicillin, 5 μg / ml of Tetracycline and Gentamicin. It inhibited *S. aureus* similarly to 40 μg / ml of Ampicillin, 10 μg / ml of Tetracycline and 20 μg / ml of Cloxacillin.

3.10.7. Comparing the activity of *Xeromphis nilotica* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* higher than 40 μg / ml of Ampicillin and similarly to10 μg / ml of Tetracycline and 10 μg / ml of Gentamicin. It inhibited *A. niger* higher than 50 μg / ml of Nystatin and10 μg / ml of Clotrimazole.

The plant methanolic extract inhibited *S. aureus* similarly to 20 μg / ml of Ampicillin, 10 μg / ml of Cloxacillin and higher than 5 μg / ml of Tetracycline. It inhibited *E. coli* similarly to 10 μg / ml of Gentamicin.

3.10.8. Comparing the activity of *Securidaca longepedunculata* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* similarly to 40 μg / ml of Ampicillin. It inhibited *A. niger* higher than 500 μg / ml of Nystatin and 40 μg / ml of Clotrimazole.

The plant methanolic extract inhibited *S. aureus* similarly to 40 μg / ml of Ampicillin, 10 μg / ml of Tetracycline and 20 μg / ml of Cloxacillin. It inhibited *E. coli* higher than 10 μg / ml of Gentamicin.

3.10.9. Comparing the activity of *Kigelia africana* extracts against standard organisms with reference drugs:

The plant chloroformic extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline and Ampicillin, and 20 μg / ml of Gentamicin. It inhibited *S. aureus* similarly to 5 μg / ml of Ampicillin and Cloxacillin. It inhibited *Ps. aeruginosa* similarly to 20 μg / ml of Tetracycline.

The plant methanolic extract inhibited *B. subtilis* similarly to 40 μg / ml of Ampicillin and inhibited *S. aureus* similarly to 40 μg / ml of Tetracycline and 10 μg / ml of Gentamicin; and higher than 40 μg / ml of Ampicillin and Cloxacillin. It inhibited *E. coli* higher than10 μg / ml of

Gentamicin and inhibited *Ps. aeruginosa* higher than 20 μg / ml Tetracycline.

3.10.10. Comparing the activity of *Courbonia virgata* extracts against standard organisms with reference drugs:

The chloroformic extract inhibited *B. subtilis* similarly to 40 μg / ml of Ampicillin. It inhibited *S. aureus* higher than 5 μg / ml of Ampicillin and Cloxacillin and similarly to 5 μg / ml of Tetracycline.

The plant methanolic extract inhibited *B. subtilis* higher than 40 μg / ml of Tetracycline and Ampicillin, and 20 μg / ml of Gentamicin. It inhibited *S. aureus* higher than 40 μg / ml of Tetracycline, Ampicillin and Cloxacillin; and10 μg / ml of Gentamicin. It inhibited *E. coli* higher than 40 μg / ml of Gentamicin, and inhibited *Ps. aeruginosa* similarly to 10 μg / ml of Tetracycline.

The aqueous extract inhibited *S. aureus* similarly to 40 μg / ml of Ampicillin, 10 μg / ml of Tetracycline and 20 μg / ml of Cloxacillin. It inhibited *Ps. aeruginosa* higher than 20 μg / ml of Tetracycline. It inhibited *A. niger* higher than 50 μg / ml of Nystatin and 20 μg / ml of Clotrimazole.

Table No. (17)

Determination of the minimum inhibitory concentrations (MICs)

Minimum inhibitory concentration (mg/ml) of crude extract against standard organisms*

Plant	Part	Solvent	B.s.	S.a.	E.c.	Pr.v.	Ps.a.	A.nig.	C.alb.
Raphanus sativus	Seeds	MeOH	75	18.75	>100	>100	>100	>100	>100
Tamarix aphylla	Leaves & Young branches	MeOH	9.38	9.38	37.5	18.75	9.38	>100	>100
Solenostemma argel	Leaves	MeOH	75	9.38	>100	>100	>100	>100	>100
Mimosa pigra	Leaves	MeOH	18.75	9.38	37.5	37.5	37.5	>100	>100
Guiera senegalensis	Leaves	MeOH	>75	9.38	>75	>75	37.5	>100	>100
Tinospora bakis	Root	MeOH	>100	>100	>100	>100	>100	>100	>100
Xeromphis nilotica	Branches	MeOH	>100	37.5	>100	>100	>100	>100	>100
Securidaca longepedunculata	Root	MeOH	>100	>100	>100	>100	>100	>100	>100
Kigelia africana	Fruits	MeOH	37.5	37.5	75	75	75	>100	>100
Courbonia virgata	Root	MeOH	75	75	>100	75	>100	>100	>100

*Standard organisms tested: B.s. = *Bacillus subtilis,*
S.a. = *Staphylococcus aureus*, E.c. = *Escherichia coli*, Pr.v. = *Proteus vulgaris*, Ps.a. = *Pseudomonas aeruginosa*, A.nig. = *Aspergillus niger*,
C.alb. = *Candida albicans*

Table No. (18)

Antibacterial and Antifungal activity of reference drugs against standard organisms

Drug	Con. µg/ml	Test organisms used*M.D.I.Z mm				
		B.s.	S.a.	E.c.	Pr.v.	Ps.a
Tetracycline	40	23	31	-	16	16
	20	21	27	-	-	13
	10	20	25	-	-	12
	5	18	17	-	-	-
Ampicillin	40	15	25	-	-	-
	20	14	20	-	-	-
	10	13	18	-	-	-
	5	12	15	-	-	-
Cloxacillin	40	-	28	-	-	-
	20	-	25	-	-	-
	10	-	20	-	-	-
	5	-	15	-	-	-
Gentamicin	40	29	35	32	25	23
	20	22	33	30	24	22
	10	20	30	17	23	22
	5	17	28	-	22	19

Drug	Con. µg/ml	A. nig	C. alb.
Nystatin	500	27	32
	50	17	28
	25	14	26
	12.5	-	23
Clotrimazole	40	30	42
	20	22	40
	10	20	31
	5	-	28

B.s. = *Bacillus subtilis*, S.a. = *Staphylococcus aureus*, E.c. = *Escherichia coli*, Pr.v. = *Proteus vulgaris*, Ps.a. = *Pseudomonas aeruginosa*, A.nig. = *Aspergillus niger*, C.alb. = *Candida albicans*
* = Mean diameter of growth inhibition zones in mm.
Average of 2 replicates
- = No inhibition zone

3.11. Susceptibility of the clinical isolates to selected plant extracts exhibiting high Antibacterial activity:

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Depending on the results of testing 30 plant extracts against the standard bacteria, the most active plant extracts were tested against 100 clinical isolates (any inactive plant extract against one of the standard bacteria, was excluded from being tested against the clinical isolates).

The 100 clinical isolates were collected randomly from the National health laboratory, Khartoum teaching hospital, Police teaching hospital and Khartoum North teaching hospital during the period from January to September 2003.18 extracts from 10 plants were tested (Tables No.20-29).

It is evident that the patterns of inhibition varied with the plant part, the solvent used for extraction and the organism tested.

Methanolic extracts showed high activity against the clinical isolates. The least activity was exhibited by the chloroformic extracts, while the aqueous extracts showed moderate activity.

The Gram positive bacteria were more susceptible than the Gram negative bacteria.

311.1. The activity of *Raphanus sativus* against clinical isolates:

None of the standard organisms were susceptible to chloroform extract, so it was not tested against the clinical isolates. Only the standard *S. aureus* was susceptible to methanolic extract, so the *S. aureus* clinical isolates were tested with the methanolic extract. All standard bacteria were susceptible to the aqueous extract, so the aqueous extract was tested against all the clinical isolates, activity of the methanolic and aqueous extracts could be due to the presence of polar compounds.

The methanolic extract exhibited high activity against seven *S. aureus* clinical isolates, intermediate activity against 12, and was inactive against eight clinical isolates.

The aqueous extract was more active than the methanolic one. Its activity varied against the 100 clinical isolates, it showed the highest activity against *S. aureus* clinical isolates and the lowest against *E.coli* clinical isolates (Table No.20, Fig. No.5).

Table No. (19)

Percentage distribution of the clinical isolates according to their sources

Organism	Urine	Ear swab	Wound	Sputum	Eye swab	Abscess
E. coli	100%	-	-	-	-	-
Pr. v.	50%	33%	16.7%	-	-	-
Ps. a.	25%	25%	50%	-	-	-
S. a.	-	40.74%	37%	7.4%	3.7%	11.11%

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

3.11.2. The activity of *Tamarix aphylla* against clinical isolates:

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Chloroform extract of this plant showed no activity against standard organisms, so it was not tested against clinical isolates.

Methanolic extract showed activity against all standard organisms, so it was tested against the 100 clinical isolates.

Aqueous extract showed activity only against *S. aureus* standard organism, so it was tested against *S. aureus* clinical isolates only.

Aqueous extract was highly active against 10 of the 27 *S. aureus* clinical isolates, and showed intermediate activity against 16 of them, and was inactive against only one of the 27 *S. aureus* clinical isolates.

Methanolic extract was very active; it was highly active against all *Ps. aeruginosa* clinical isolates, and least active against *S. aureus* clinical isolates (Table No.21, Fig. No.6).

Activity of the methanolic and aqueous extracts could be due to the presence of polar compounds.

3.11.3. The activity of *Solenostemma argel* against clinical isolates:

Depending on the standard bacterial results, the chloroform extract was not tested against the clinical isolates.

The methanolic extract showed high activity against *S. aureus* and *Pr. vulgaris.* Standard organisms, so it was tested against both *S. aureus* and *Pr. vulgaris.* clinical isolates.

Aqueous extract showed activity only against *S. aureus* standard organism, so it was tested against *S. aureus* clinical isolates only.

Aqueous extract was highly active against only one *S. aureus* clinical isolate, intermediately active against three of them, and inactive against 23 *S. aureus* clinical isolates, generally revealing a low activity generally.

The methanolic extract showed high activity against *Pr. vulgaris* clinical isolates, with only one *Pr. vulgaris* exhibiting resistance.

10 *S. aureus* clinical isolates showed high sensitivity against MeOH, 12 showed intermediate activity, and only five were resistant to the MeOH extract (Table No.22, Fig. No.7).

Activity of the methanolic and aqueous extracts could be due to the presence of polar compounds.

3.11.4. The activity of *Mimosa pigra* against clinical isolates:

The chloroformic and aqueous extracts of this plant against standard organisms were inactive, so both extracts were excluded.

The methanolic extract was active against all standard organisms, so it was tested against the 100 clinical isolates, this activity could be due to the presence of polar compounds.

The methanolic extract was active against all *S. aureus* and *E. coli* clinical isolates, and against 36 of the *Ps. aeruginosa* clinical isolates and moderately active against the remaining four *Ps. aeruginosa* clinical isolates.

The methanolic extract was highly active against 15 *Pr. vulgaris* clinical isolates, moderately active against two of them and inactive against only one *Pr. vulgaris* clinical isolate.

It is evident that the methanolic extract of this plant did show remarkably high activity (Table No.23, Fig. No.8).

3.11.5. The activity of *Guiera senegalensis* against clinical isolates:

The methanolic and aqueous extracts of this plant were active against standard organisms, which could be due to the presence of polar compounds, so both extracts were tested against all the clinical isolates.

The chloroformic extract was inactive against standard organisms, so it was not tested against the clinical isolates.

The methanolic and aqueous extracts were highly active against the 100 clinical isolates, the results were nearly similar, but the methanolic extract was stronger.

The methanolic extract was highly active against all *S. aureus* clinical isolates except one, and it was highly active against all *E. coli* clinical isolates, highly active against all *Ps. aeruginosa* clinical isolates and highly active against the *Pr. vulgaris* clinical isolates except one.

Aqueous extract was highly active against 21 *S. aureus* clinical isolates, and moderately active against six of them.

Aqueous extract was highly active against all *E. coli* clinical isolates, and highly active against 37 of the *Ps. aeruginosa* clinical isolates, moderately active against three *Ps. aeruginosa* clinical isolates. It was also highly active against 15 *Pr. vulgaris* clinical isolates, moderately active against two *Pr. vulgaris* clinical isolates and inactive against only one *Pr. vulgaris* clinical isolate (Table No.24, Fig. No.9).

3.11.6. The activity of *Tinospora bakis* against clinical isolates:

The chloroformic and aqueous extracts of this plant were active against standard organisms, so both extracts were not subjected to test against the clinical isolates.

The methanolic extract showed activity against *S. aureus* and *Pr. vulgaris* standard organisms only, so it was tested against *S. aureus* and *Pr. vulgaris* clinical isolates only.

The methanolic extract was nearly similarly highly active against both against *S. aureus* and *Pr. vulgaris* clinical isolates (Fig. No.15), but *S. aureus* clinical isolates were more susceptible.

One (11.11%) of the *Pr. vulgaris* clinical isolates were resistant to the methanolic extract, whereas only 3.7% of the *S. aureus* clinical isolates were resistant to it (Table No.25, Fig. No.10).

Activity of the methanolic extract could be due to the presence of polar compounds.

3.11.7. The activity of *Xeromphis nilotica* against clinical isolates:

The chloroformic and aqueous extracts of this plant were active against standard organisms, so both extracts were not subjected to test against the clinical isolates.

The methanolic extract was active against *S. aureus*, E. *coli* and *Pr. vulgaris* standard organisms, so it was tested against their clinical isolates, that could be due to the presence of polar compounds.
The most sensitive and least resistant clinical isolates to the methanolic extract were those of *S. aureus*, where the extract showed high activity

against nine *S. aureus* clinical isolates, moderate activity against 10 of them and no activity against eight *S. aureus* clinical isolates.

The lowest susceptible clinical isolates were those of *Pr. vulgaris*, which exhibited the highest percentage of resistant clinical isolates (44.44%) to the methanolic extract (Table No.26, Fig. No.11).

3.11.8. The activity of *Securidaca longepedunculata* against clinical isolates:

The chloroformic and aqueous extracts of this plant showed no activity against standard organisms and consequently both extracts were not examined against the clinical isolates.

On the other hand, the methanolic extract showed activity against *S. aureus* and *E. coli* standard organisms, which could be due to the presence of polar compounds, so it was tested against their clinical isolates.

The methanolic extract was more active against *S. aureus* clinical isolates than *E. coli* clinical isolates (Table No.27, Fig. No.12).

3.11.9. The activity of *Kigelia africana* against clinical isolates:

Methanolic extract was the most active extract. Chloroformic extract showed medium activity, and the aqueous extract was the lowest.

Methanolic extract exhibited high, moderate and inactivity against equal number of *S. aureus* clinical isolates (nine clinical isolates for each), while it highly inhibited the growth of six *E. coli* clinical isolates and 12 *Ps. aeruginosa* clinical isolates.

Chloroformic extract showed higher activity against *Pr. vulgaris* clinical isolates than that against *S. aureus* clinical isolates.

Aqueous extract was active only against *S. aureus* clinical isolates, where it had high activity against four *S. aureus* clinical isolates, moderate activity against eight of them and inactivity against 15 *S. aureus* clinical isolates (Table No.28, Fig. No.13).

3.11.10. The activity of *Courbonia virgata* against clinical isolates:

The chloroformic extract showed the lowest activity, where it showed moderate activity against 16 *S. aureus* clinical isolates, and was inactive against 11 of them. The aqueous extract exhibited the highest activity against both *S. aureus* and *Ps. aeruginosa* clinical isolates than the activity showed by the methanolic extract. *Ps. aeruginosa* clinical isolates were the most susceptible to the plant extracts. *S. aureus* clinical isolates were the least susceptible to the plant extracts (Table No.29, Fig. No.14).

Table No. (20)

The activity of *Raphanus sativus* seeds against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	**Intermediate**	**Resistant**
S. aureus	MeOH	7	12	8
	H₂O	9	15	3
E. coli	H₂O	5	4	6
Ps. aeruginosa	H₂O	20	11	9
Pr. vulgaris	H₂O	9	6	3

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)　　　　　Gram (-) bacteria *(E.c., Pr.v., Ps.a.)*

>18 mm (MIZD) = sensitive　　　　 >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate　 13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant　　　　< 13 mm (MIZD) = resistant
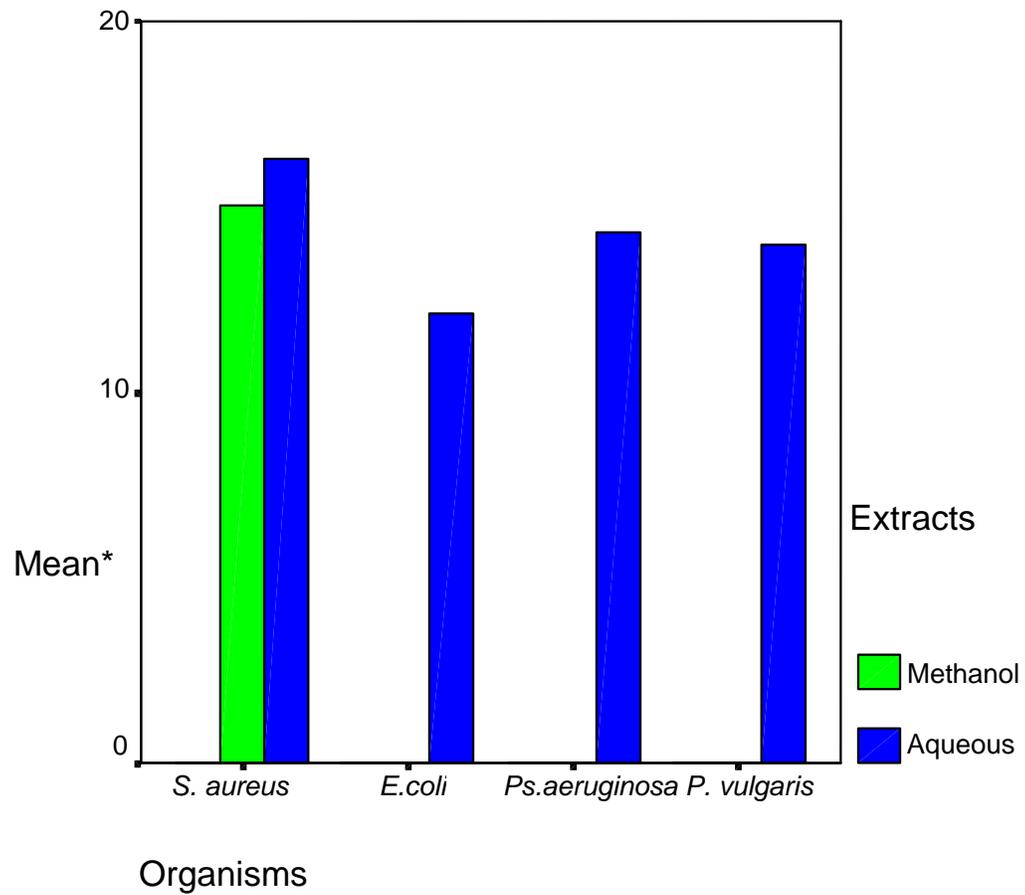
*Mean inhibition zone diameter (mm)

Fig. No. (5)

The activity of *Raphanus sativus* seeds against clinical isolates

Table No. (21)

The activity of *Tamarix aphylla* leaves against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	**Intermediate**	**Resistant**
S. aureus	MeOH	20	5	2
	H₂O	10	16	1
E. coli	MeOH	14	1	0
Ps. aeruginosa	MeOH	40	0	0
Pr. vulgaris	MeOH	16	1	1

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c., Pr.v., Ps.a.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
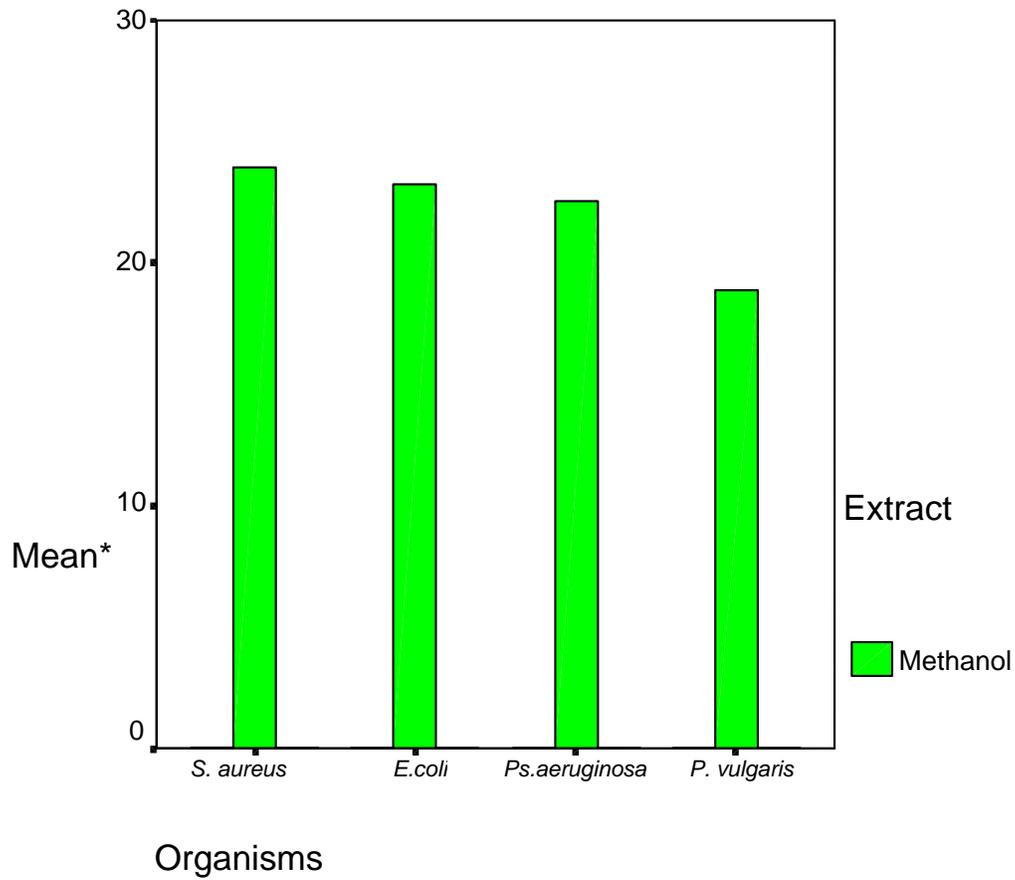14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant
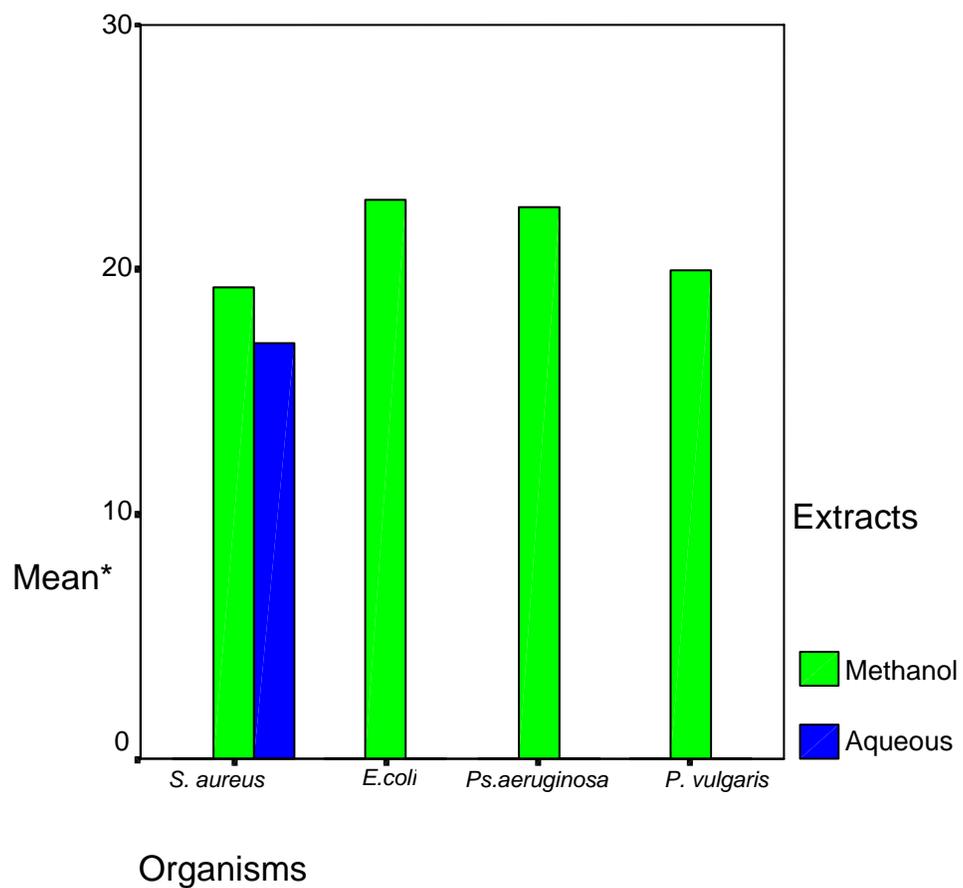
*Mean inhibition zone diameter (mm)

Fig. No. (6)

The activity of *Tamarix aphylla* leaves against clinical isolates

Table No. (22)

The activity of *Solenostemma argel* leaves against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	10	12	5
	H$_2$O	1	3	23
Pr. vulgaris	MeOH	15	2	1

Total No. of clinical isolates = 45
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria (*Pr.v.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant
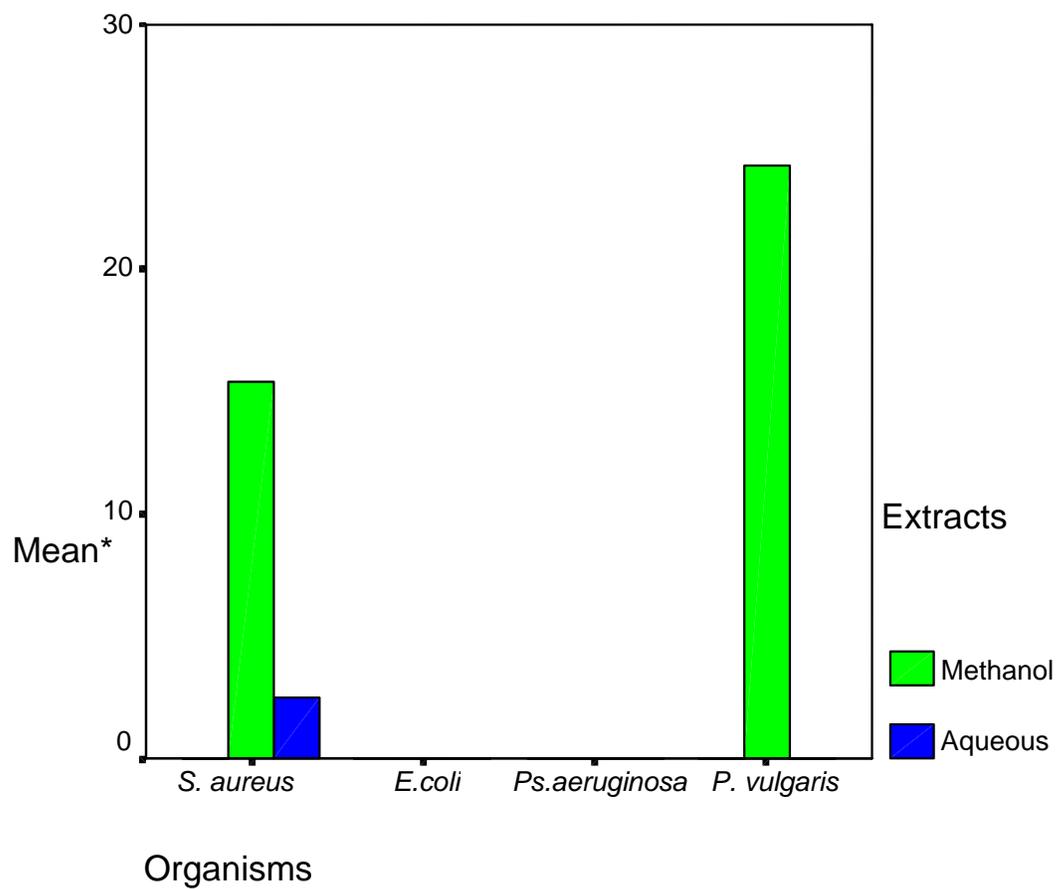
Fig. No. (7)

The activity of *Solenostemma argel* leaves against clinical isolates

Table No. (23)

The activity of *Mimosa pigra* leaves against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	27	0	0
E. coli	MeOH	15	0	0
Ps. aeruginosa	MeOH	36	4	0
Pr. vulgaris	MeOH	15	2	1

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c., Pr.v., Ps.a.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant

30 —

20 —

Mean*

10 —

0 —

S. aureus   E.coli   Ps.aeruginosa   P. vulgaris

Extract

☐ Methanol

Organisms

*Mean inhibition zone diameter (mm)

Fig. No. (8)

The activity of *Mimosa pigra* leaves against clinical isolates

Table No. (24)

The activity of *Guiera senegalensis* leaves against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	26	0	1
	H_2O	21	6	0
E. coli	MeOH	15	0	0
	H_2O	15	0	0
Ps. aeruginosa	MeOH	40	0	0
	H_2O	37	3	0
Pr. vulgaris	MeOH	17	0	1
	H_2O	15	2	1

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c., Pr.v., Ps.a.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant
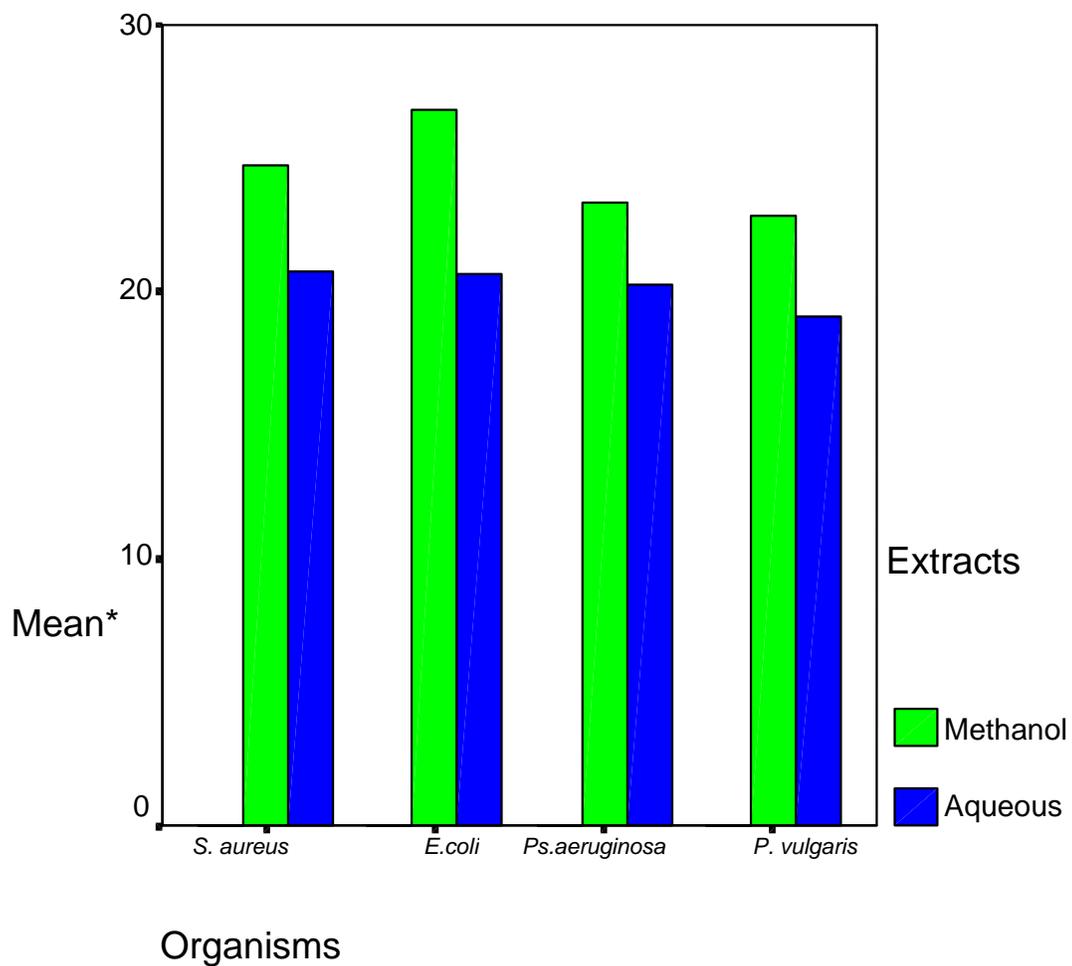
*Mean inhibition zone diameter (mm)

Fig. No. (9)

The activity of *Guiera senegalensis* leaves against clinical isolates

Table No. (25)

The activity of *Tinospora bakis* Root against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	17	9	1
Pr. vulgaris	MeOH	11	5	2

Total No. of clinical isolates = 45
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria (*Pr.v.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant
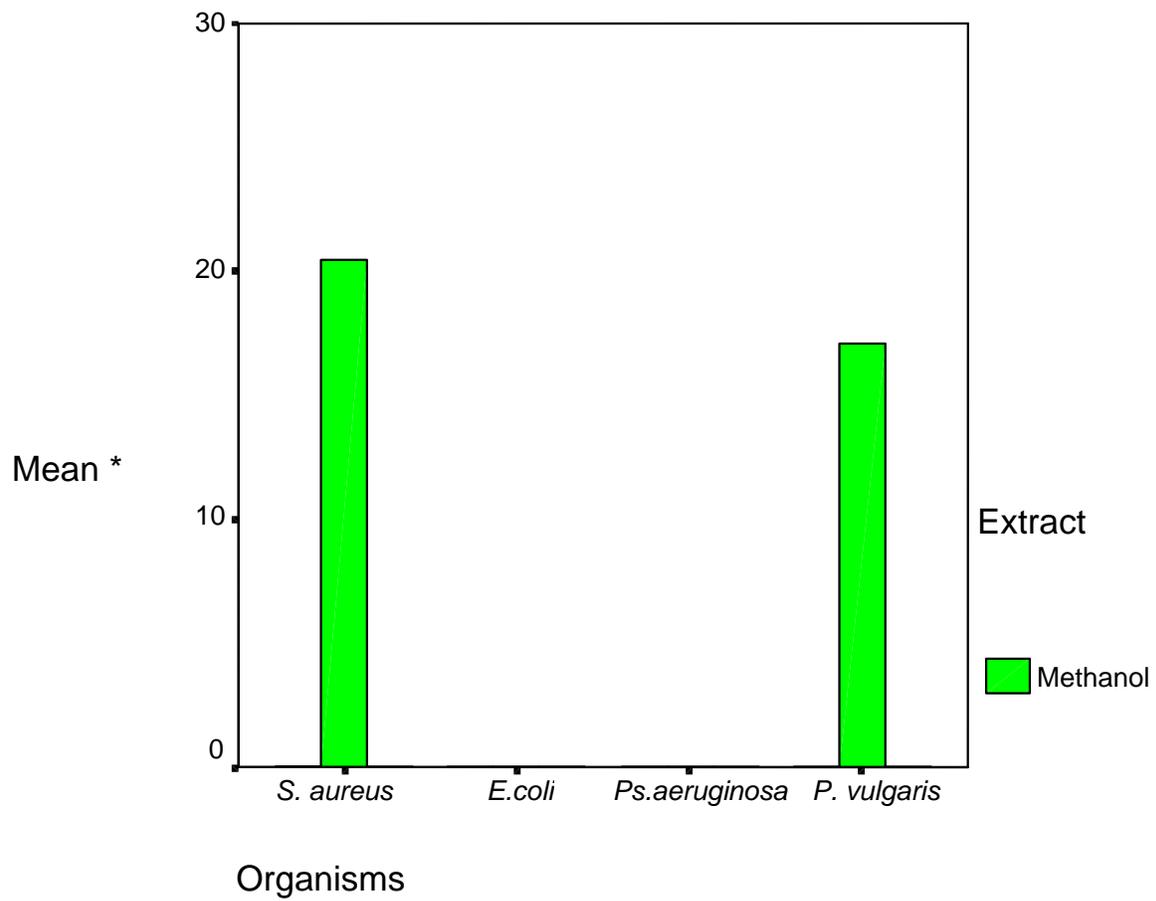
*Mean inhibition zone diameter

Fig. No. (10)

The activity of *Tinospora bakis* Root against clinical isolates

Table No. (26)

The activity of *Xeromphis nilotica* branches against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	9	10	8
E. coli	MeOH	1	9	5
Pr. vulgaris	MeOH	2	8	8

Total No. of clinical isolates = 60
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c., Pr.v.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate 13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant
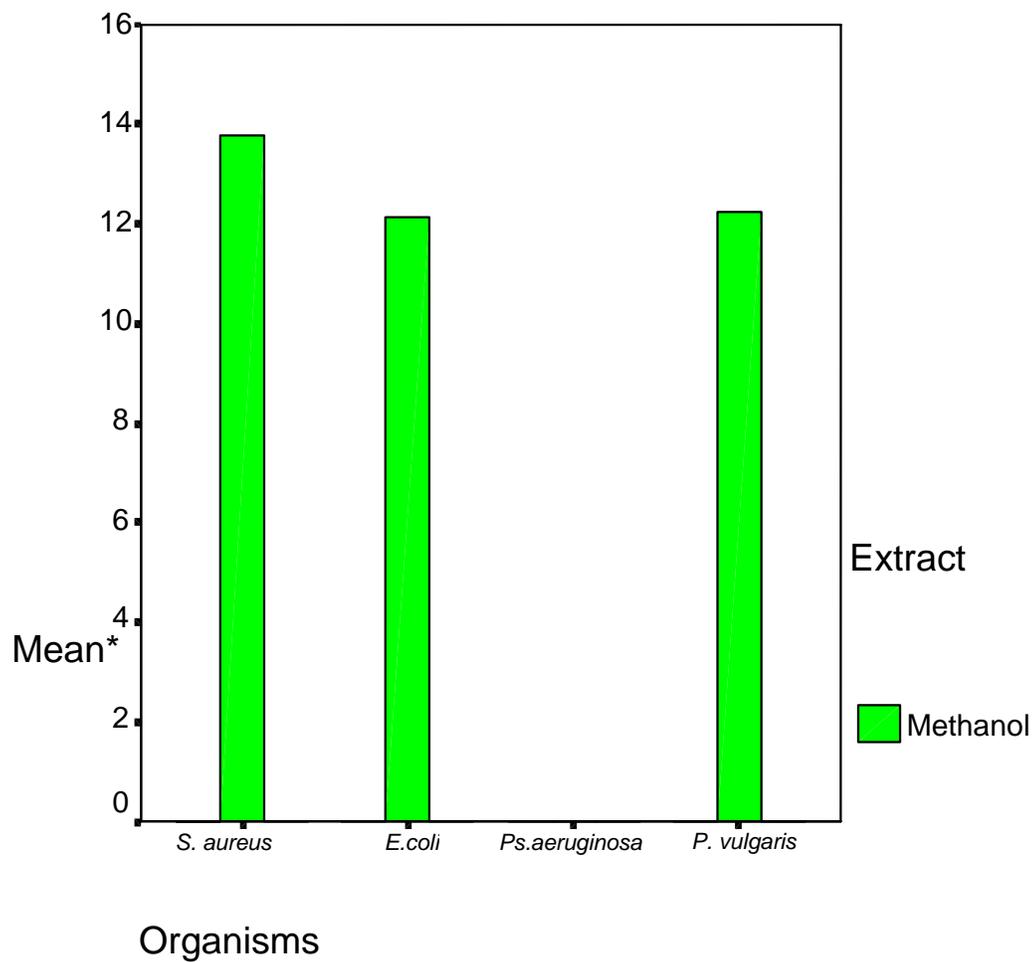
Organisms

*Mean inhibition zone diameter (mm)

Fig. No. (11)

The activity of *Xeromphis nilotica* branches against clinical isolates

Table No. (27)

The activity of *Securidaca longepedunculata* Root against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	Intermediate	Resistant
S. aureus	MeOH	6	12	9
E. coli	MeOH	3	3	9

Total No. of clinical isolates = 42
No. of *Escherichia coli* clinical isolates = 15
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c..)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant

Mean*

Organisms

Extract

Methanol
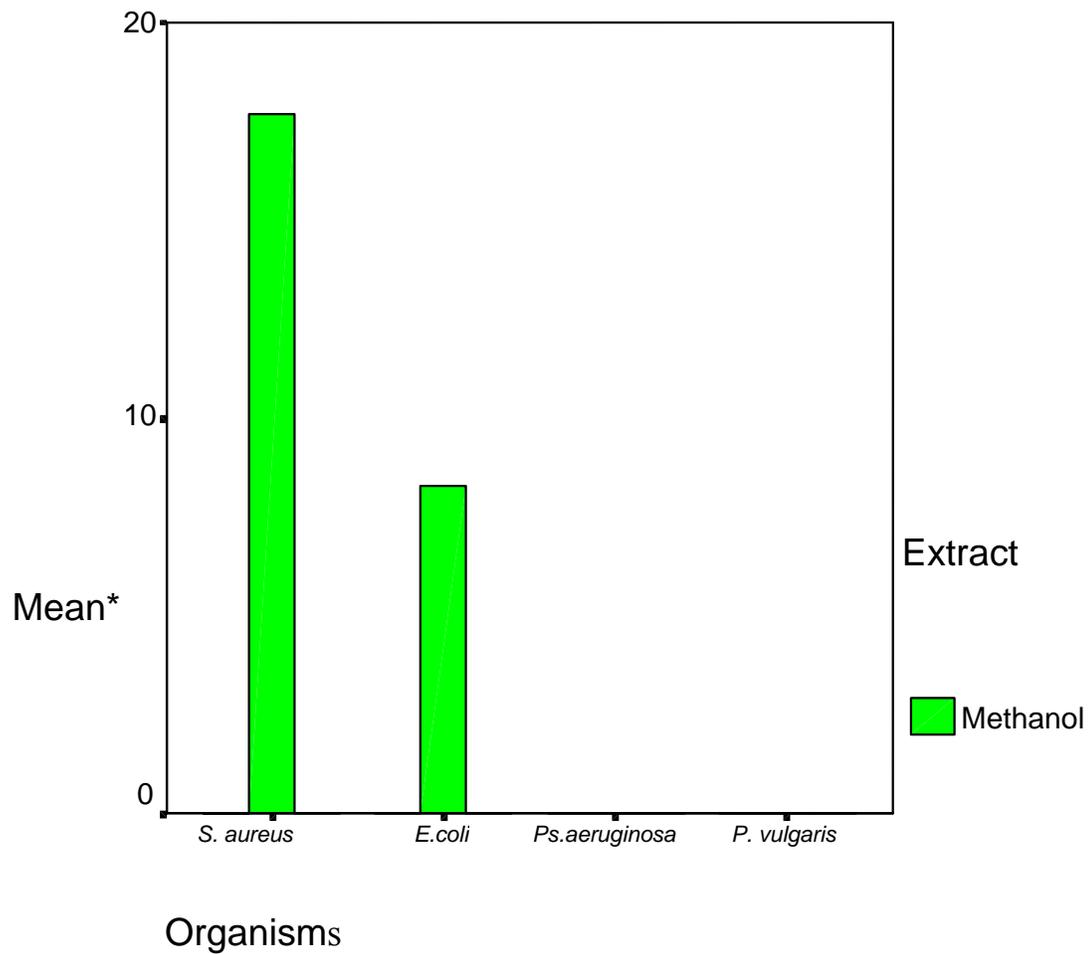
*Mean inhibition zone diameter (mm)

Fig No. (12)

The activity of _Securidaca longepedunculata_ Root against clinical isolates

Table No. (28)

The activity of *Kigelia africana* fruit against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	**Intermediate**	**Resistant**
S. aureus	CHCl$_3$	2	18	7
	MeOH	9	9	9
	H$_2$O	4	8	15
E. coli	MeOH	6	5	4
Ps. aeruginosa	MeOH	12	23	5
Pr. vulgaris	CHCl$_3$	6	11	1

Total No. of clinical isolates = 100
No. of *Escherichia coli* clinical isolates = 15
No. of *Proteus vulgaris* clinical isolates = 18
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)　　　　　Gram (-) bacteria *(E.c., Pr.v., Ps.a.)*
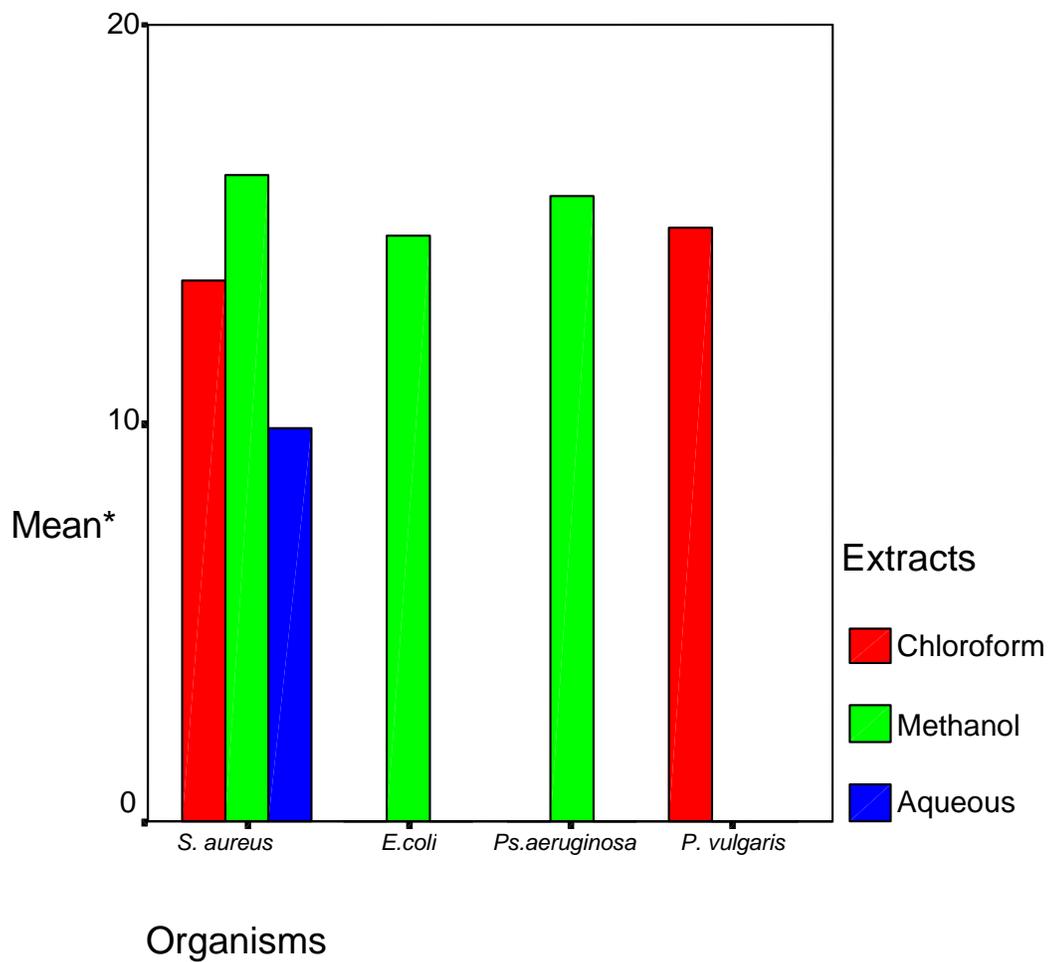
>18 mm (MIZD) = sensitive　　　 >16 mm (MIZD) = sensitive
14-18 mm (MIZD) = intermediate　13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant　　　< 13 mm (MIZD) = resistant

*Mean inhibition zone diameter (mm)

Fig. No. (13)

The activity of *Kigelia africana* fruit against clinical isolates

Table No. (29)

The activity of *Courbonia virgata* Root against clinical isolates

Organism tested	Solvent	No. of clinical isolates		
		Sensitive	**Intermediate**	**Resistant**
S. aureus	CHCl$_3$	0	16	11
	MeOH	6	14	7
	H$_2$O	7	9	11
E. coli	MeOH	6	5	4
Ps. aeruginosa	H$_2$O	26	9	5

Total No. of clinical isolates = 88
No. of *Escherichia coli* clinical isolates = 15
No. of *Pseudomonas aeruginosa* clinical isolates = 40
No. of *Staphylococcus aureus* clinical isolates = 27

Interpretation of sensitivity results:

Gram (+) bacteria (*S. a.*)   Gram (-) bacteria *(E.c., Ps.a.)*

>18 mm (MIZD) = sensitive   >16 mm (MIZD) = sensitive
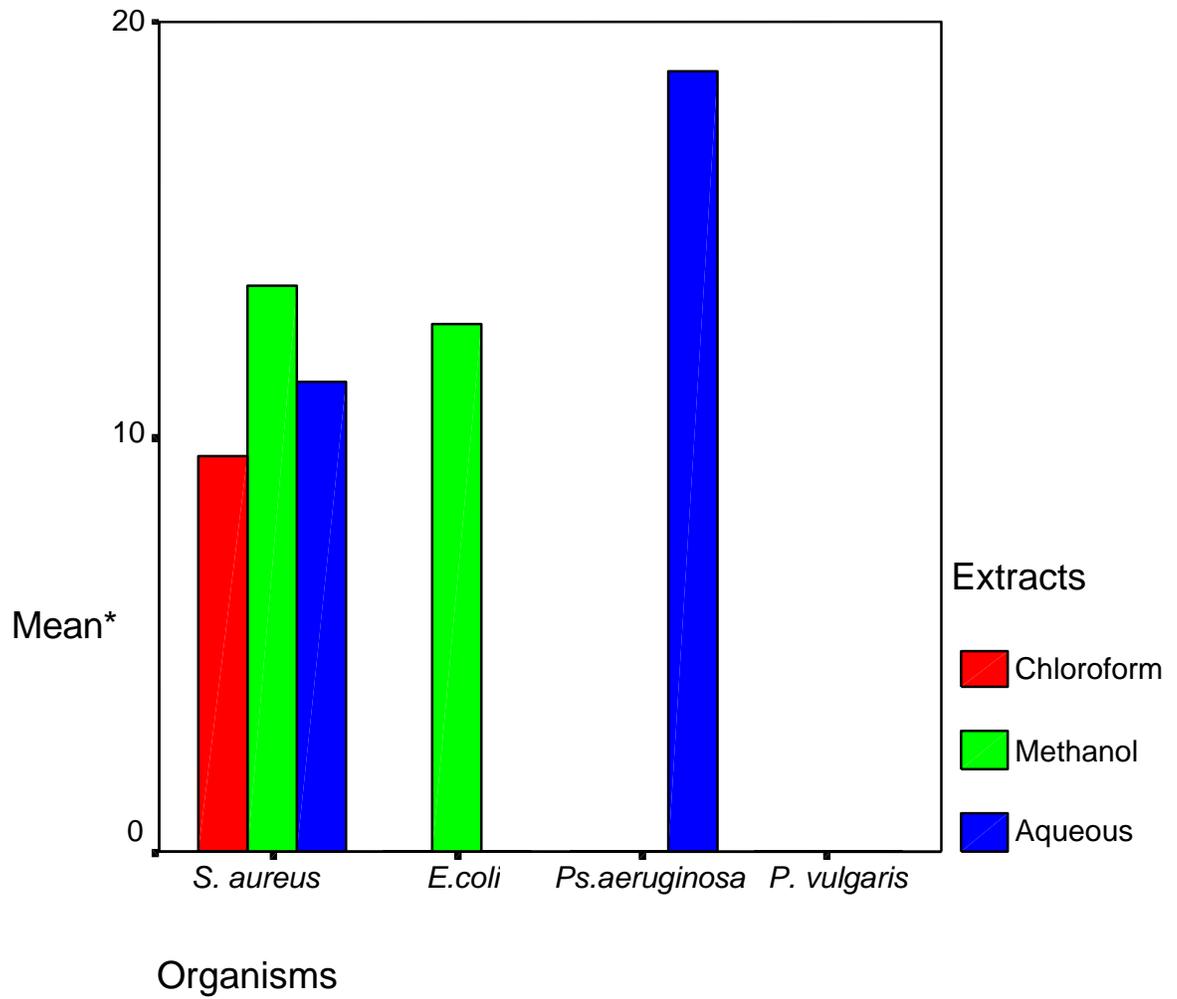14-18 mm (MIZD) = intermediate   13-16 mm (MIZD) = intermediate
< 14 mm (MIZD) = resistant   < 13 mm (MIZD) = resistant

*Mean inhibition zone diameter (mm)

Fig. No. (14)

The activity of *Courbonia virgata* Root against clinical isolates

Fig. No. (15)
The *In vitro* activity of *Tinospora bakis* root methanolic extract against *Proteus vulgaris* clinical isolate No. 8

Fig. No. (16)
The *In vitro* activity of *Securidaca longepedunculata* root methanolic (upper cups) & chloroformic extracts (lower cups) against standard *Aspergillus niger*

3.12. *In vitro* and *in vivo* activity results of *Solenostemma argel* leaves:

3.12.1. *In vitro* antibacterial activity results of *Solenostemma argel* leaves:

Referring to the results obtained in table No. (7), *Solenostemma argel* leaves methanolic extract showed remarkable high *in vitro* activity against some of the tested Gram positive and Gram negative bacteria (i.e.*Staphylococcus aureus* and *Proteus vulgaris* standard organism).

That activity was confirmed by testing the plant methanolic extract *in vitro* against 27 *Staphylococcus aureus* and 18 *Proteus vulgaris* clinical isolates (Table No.22, Fig. No.7).

3.12.2. *Solenostemma argel* leaves as a traditional wound healing stimulant:

Solenostemma argel is used in Sudanese folkloric medicine in three forms:

1- Internally, in the form of hot water decoction of macerated water extract, as an antispasmodic and in the treatment of GIT disturbances, hypercholesterolemia and diabetes mellitus.

2- Externally, in poultice form, as anti-inflammatory and antirheumatic.

3- Inhalation of its smoke in the treatment of measles and cold (Shayoub, 2003).

Elkamali (1997) also reported that *Solenostemma argel* fine powdered leaves are used traditionally as a wound healing agent in Sudan, Egypt, Libya, Sudia Arabia and Algeria.

The literature survey revealed that no previous studies on the *in vivo* antimicrobial and wound healing effect of *Solenostemma argel* have been done, and due to its antimicrobial activity, this study was an attempt to evaluate the wound healing and antimicrobial activity of *Solenostemma argel* on rats using the prepared Polyethylene Glycol ointment.

3.12.3. Results of testing the *in vitro* activity of the prepared Ointments:

Four different ointments containing *Solenostemma argel* methanolic extract were tested using the cup-plate agar diffusion method. Only the ointment prepared with Polyethylene Glycol (PEG) base, readily diffused in nutrient agar, the rest did not.

Using the PEG ointment containing 1%, 2% and 5% methanolic extract of *Solenostemma argel* leaves, gave 18 mm, 20 mm and 22 mm as an average of the inhibition zones, respectively, against the standard *S. aureus* (Table No.30).

The result of testing the antibacterial activity of the pure four bases as controls (without including the plant extract), was negative, showing that the bases alone did not have the ability to suppress microbes.

These results justified using the PEG ointment as a vehicle, which was able to release the plant extract, to be applied topically on rats.

PEG containing 2% of *Solenostemma argel* methanolic extract was selected for studying the wound healing.

3.12.4. Result of the first *in vivo* trial: Wound healing activity of *Solenostemma argel* (non-infected rats):

Results were obtained by measuring the wound healing percentage of the three groups of rats in this trial (Group 1: wound only, group 2: wound + Tetracycline 3% ointment, group 3: wound + PEG containing '*S. argel*' 2% ointment) as shown in table No. (31).

3.12.4.1. Interpretation of result of the first *in vivo* trial:

In the first (wounded/ not treated) group, healing was completed in 11 days. In the second (wounded / treated with Tetracycline 3% ointment) group, 10 days were required for the completion of healing. In the third (wounded / treated with PEG containing '*S. argel*' 2% ointment) group, the healing period was reduced to 9 days (Fig No.17; 19, 20, 21, 22, 23, 24).

Significant differences ($p < 0.05$) between PEG containing '*S. argel*' 2% ointment and Tetracycline 3% ointment groups were observed at the first day of treatment until the end of the treatment course.

Significant differences (p=0.001) between groups was calculated in table No. (33-i).

Comparing the means showed significant differences when comparing the total means with the means of the third group, but comparing the total means with the means of the first and second groups showed insignificant results, elucidating the activity of the PEG containing
'S. argel' 2% ointment (Table No.33-ii).

3.12.5 Result of the second *in vivo* trial: Wound healing activity of *Solenostemma argel* (infected rats):

Results were obtained by measuring the wound healing percentage of the three groups of rats in this trail (Group 1: wound only, group 2: wound + Tetracycline 3% ointment, group 3: wound + PEG containing *'S. argel'* 2% ointment) as shown in table No. (32).

3.12.5.1. Interpretation of result of the second *in vivo* trial:

In the first (wounded/ infected) group, healing was completed in 11 days, whereas in the second (wounded / treated with Tetracycline 3% ointment / infected) group, 10 days were required for the completion of healing. In the third (wounded / treated with PEG containing *'S. argel'* 2% ointment/ infected) group, the healing period was reduced to 9 days (Fig. No.18).

Infection was done artificially with standard *S. aureus*.

Insignificant differences (p>0.05) between groups were calculated in table No. (34-i). Comparing the means showed significant differences

When comparing the total means with the means of the first group, but comparing the total means with the means of the second and third groups showed insignificant results, showing that the PEG ointment containing *Solenostemma argel* in 2% concentration, had wound healing activity but it had no *in vivo* antibacterial activity when using this concentration. (Table No.34-ii).

Table No. (30)

Results of testing the *in vitro* activity of Ointments

ointment⟍conc.	Vaseline	E.wax	Lanolin	PEG
1%	-	-	-	18 mm*
2%	-	-	-	20 mm*
5%	-	-	-	22 mm*

(-): no inhibition

*Mean diameter of inhibition zones, in mm, average of 2 replicates

Organism tested: *S. aureus*.

3.12.6. Discussion of the two wound healing experiments:

Healing of wound involves various phases such as inflammation, coagulation, collagenation, wound contraction and epithelisation, while the phases between coagulation and collagenation are interlinked, the phases of wound contraction and epithelization are completely independent from each other but run concurrently (Bairy and Rao 2001).

In this study excision wound model for contraction and epithelization was employed, and the effect of *S. argel* methanolic extract was investigated in open skin wounds on rats.

The results of the present study indicated that the methanolic extract of *S. argel* was able to reduce the time required for wound healing, in both infected and non infected wounds, but it had no significant *in vivo* antibacterial activity when using the plant extract in this concentration.

The results were obtained from the faster contraction of wound treated with *S. argel* extract in comparison with Tetracycline 3% ointment or untreated groups.

S. argel methanolic extract, was more potent (faster) than Tetracycline 3% ointment as standard healing agent.

The percentage of healing with PEG containing *'S. argel'* 2% ointment was significantly higher ($p<0.05$) than Tetracycline group from the first day of treatment until the closure of wounds in the first experiment.

In the second experiment the percentage of healing with PEG containing *'S. argel'* 2% ointment was significantly lower ($p<0.05$) than Tetracycline group.

Although some studies have been done regarding the pharmacological properties of *S. argel*, however, wound healing effect of this plant has not been very well documented.

Studies have been done to elucidate the mechanisms of the action of *S. argel* spasmolytic and uterine relaxant activities on the pregnant and non pregnant rat uterus; it was explained on the basis of its local anesthetic effect (El Tahir *et al*, 1987).

However more studies are required to elucidate the exact mechanism of *S. argel* in wound healing models.

In the case of successful clinical trials it may be employed as a healing agent or can be used in conjunction with other healing drugs to obtain a better healing profile.

Table No. (31)

First *in vivo* trial: Percentage wound healing activity of *Solenostemma argel* (non-infected rats)

Day	Group 1 (wound/untreated)					Group 2 (wound+Tetracycline 3%)					Group 3 (wound+ *S. argel* 2% PEG oint.)				
	Rat1	Rat2	Rat3	Rat4	Rat5	Rat1	Rat2	Rat3	Rat4	Rat5	Rat1	Rat2	Rat3	Rat4	Rat5
0	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
1	18.75%	28.58%	25%	20%	25%	36.37%	37.5%	25%	37.5%	11.12%	28.58%	30%	45%	31.82%	50%
2	43.75%	42.86%	35%	45%	45%	36.37%	37.5%	6.25%	25%	22.23%	21.43%	30%	35%	31.82%	45%
3	43.75%	50%	45%	55%	50%	50%	37.5%	31.25%	43.75%	38.89%	50%	35%	45%	40.91%	65%
4	25%	50%	45%	55%	60%	59.10%	43.25%	37.5%	56.25%	44.45%	50%	50%	60%	45.46%	65%
5	25%	50%	45%	55%	60%	59.10%	43.25%	62.5%	56.25%	44.45%	50%	50%	60%	45.46%	65%
6	56.25%	78.58%	65%	70%	70%	68.19%	75%	75%	62.5%	61.12%	71.43%	75%	75%	59.10%	80%
7	62.50%	78.58%	70%	70%	80%	81.82%	75%	93.75%	62.5%	66.67%	78.58%	75%	75%	72.73%	90%
8	81.25%	100%	75%	80%	90%	81.82%	87.5%	100%	87.5%	72.23%	100%	100%	100%	81.82%	100%
9	100%	100%	90%	80%	100%	100%	100%	100%	93.75%	100%	100%	100%	100%	100%	100%
10	100%	100%	100%	95%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
11	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table No. (32)

Second *in vivo* trial: Percentage wound healing activity of *Solenostemma argel* (infected rats)

Day	Group 1 (wound/infected)					Group 2 (wound+ infection+Tetr. 3%)					Group 3 (wound+inf.+ *S. argel* 2% PEG oint.)				
	Rat1	Rat2	Rat3	Rat4	Rat5	Rat1	Rat2	Rat3	Rat4	Rat5	Rat1	Rat2	Rat3	Rat4	Rat5
0	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
1	40.91%	35.72%	50%	10%	50%	35%	40%	20%	37.5%	18.19%	31.82%	33.34%	40%	50%	22.73%
2	50%	42.86%	44.45%	30%	50%	50%	30%	40%	43.75%	45.46%	54.55%	33.34%	50%	55%	36.37%
3	54.55%	50%	55.56%	30%	55%	30%	35%	65%	50%	45.46%	54.55%	29.17%	45%	55%	31.82%
4	54.55%	50%	55.56%	60%	60%	50%	45%	55%	75%	45.46%	50%	33.34%	70%	60%	36.37%
5	54.55%	50%	55.56%	60%	60%	50%	45%	55%	75%	45.46%	50%	33.34%	70%	60%	36.37%
6	68.19%	71.43%	66.67%	70%	70%	60%	60%	75%	81.25%	72.73%	68.19%	58.34%	80%	70%	72.73%
7	72.73%	85.72%	77.78%	70%	80%	75%	70%	75%	81.25%	81.82%	72.73%	66.67%	80%	85%	72.73%
8	86.37%	92.86%	88.89%	90%	80%	100%	80%	100%	100%	81.82%	86.37%	75%	95%	100%	100%
9	93.19%	92.86%	100%	100%	90%	100%	95%	100%	100%	100%	100%	100%	100%	100%	100%
10	100%	100%	100%	100%	95%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
11	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

Table No. (33)

i-Univariate Analysis of Variance *of the first *in vivo* trial (Non-infected rats)

Tests of Between-Subjects Effects

Dependent Variable: Wound area(cm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	171785.801[a]	35	4908.166	88.337	.000
Intercept	679350.687	1	679350.687	12226.869	.000
GROUP	790.565	2	395.282	7.114	.001
DAY	169345.528	11	15395.048	277.078	.000
GROUP * DAY	1649.709	22	74.987	1.350	.150
Error	8000.945	144	55.562		
Total	859137.433	180			
Corrected Total	179786.746	179			

a. R Squared = .955 (Adjusted R Squared = .945)

ii-Means*

Report

Wound area(cm)

Groups	Mean	N	Std. Deviation
wd	60.5808	60	31.0966
wd+Tr	59.4030	60	32.4423
Wd +Oin	64.3190	60	31.8487
Total	61.4343	180	31.6922

*SPSS programme ver.10 was used for the statistical calculations

Table No. (34)

i-Univariate Analysis of Variance *of the second *in vivo* trial (Infected rats)

Tests of Between-Subjects Effects

Dependent Variable: Wound area(per%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	158988.926[a]	35	4542.541	64.584	.000
Intercept	731018.790	1	731018.790	10393.289	.000
GROUP	19.513	2	9.756	.139	.871
DAY	158385.639	11	14398.694	204.714	.000
GROUP * DAY	583.774	22	26.535	.377	.995
Error	10128.334	144	70.336		
Total	900136.049	180			
Corrected Total	169117.260	179			

a. R Squared = .940 (Adjusted R Squared = .926)

ii-Means*

Report

Wound area(per%)

Groups	Mean	N	Std. Deviation
Wound+Inf	64.1827	60	29.5478
Wound+Inf+Tr	63.5858	60	31.6321
Wound+Inf+Oir	63.4145	60	31.5024
Total	63.7277	180	30.7374

*SPSS programme ver.10 was used for the statistical calculations
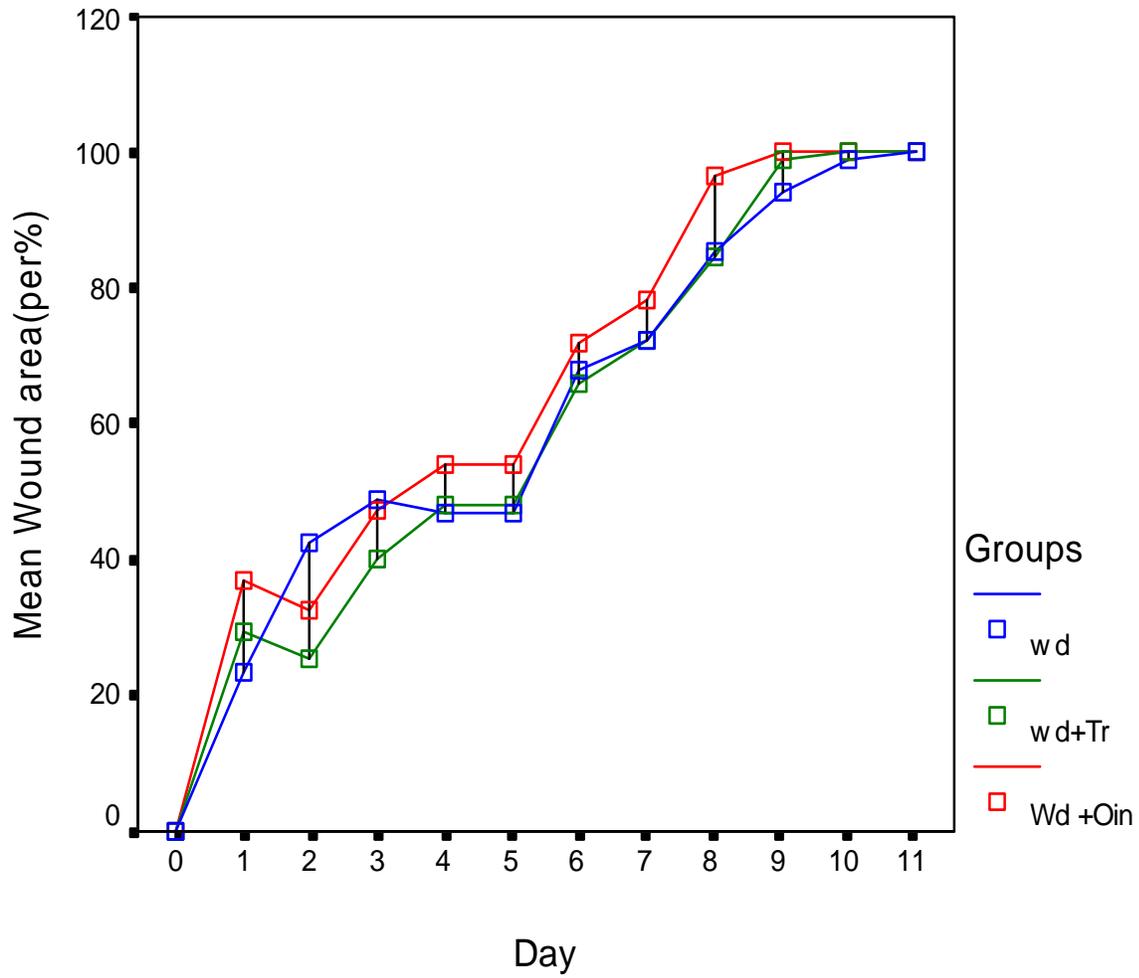
Fig.No. (17)
First *in vivo* trial: Percentage wound healing activity of
***Solenostemma argel* (non-infected rats)**
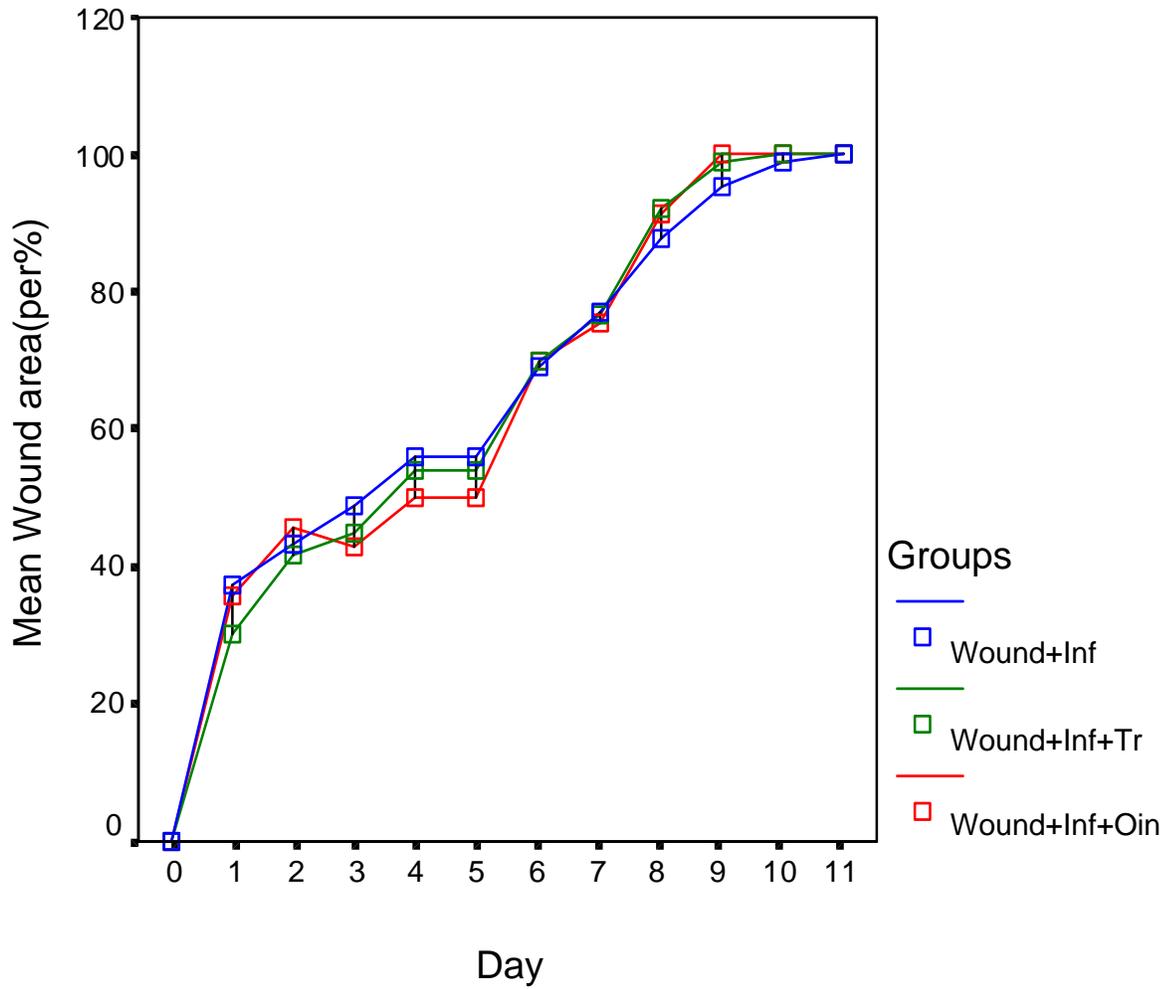
Fig.No. (18)
Second *in vivo* trial: Percentage wound healing activity of
***Solenostemma argel* (infected rats)**

Fig. No. (19)
First *in vivo* trial (day zero) Wound length (1 cm) in one of the 3rd group non-infected rats (treated with *S. argel* 2% PEG Oint.)

Fig.No. (20)
First *in vivo* trial (day zero): Wound length (1cm) in one of the 1st Group non-infected rats (untreated)

Fig.No. (21)
First *in vivo* trial (day five): Wound length (0.5 cm) in one of the 3rd
Group non-infected rats (treated with *S. argel* 2% PEG oint.)

Fig.No. (22)
First *in vivo* trial (day five): Wound length (0.75 cm) in one of the 1st
Group non-infected rats (untreated)

Fig.No. (23)
First *in vivo* trial (day nine): Full healing in one of the 3rd Group non-infected rats (treated with *S. argel* 2% PEG oint.)

Fig.No. (24)
First *in vivo* trial (day nine): Wound length (0.2 cm) in one of the 1st Group non-infected rats (untreated)

3.13. CONCLUSIONS

1- Availability and cost of antimicrobial drugs are a real problem for many people in Sudan. Development of resistance to many drugs in current use is another problem that emphasized the need for urgent search for new and cheap antimicrobial drugs.

2- Sudan has huge resources of medicinal plants. Exploitation of this medicinal flora represents an important means of obtaining cheap and effective drugs necessary to resolve the health problems in the Sudan.

3- The results of the present work indicate that there are promising plants with high and broad antimicrobial activity, when compared with some antimicrobial drugs in current use. This verified the claimed bioactivity of these plants employed in traditional medicine in Sudan.

4- *Solenostemma argel*, which is a native plant and available in every Sudanese house, proved to have wound healing activity, and this justifies its traditional use as a wound healing agent.

3.14. SUGGESTIONS FOR FUTURE WORK

1- Pharmacological, toxicological and clinical studies should be carried out on the selected medicinal plants (especially *Solenostemma argel*) to assess their safety, therapeutic efficacy and potential for commercial utilization.

2- Formulation of the active extracts and/or principles in suitable dosage forms, with special reference to *Solenostemma argel* leaves which proved to be a potent wound healing agent.

3- Bio-assay-guided fractionation and purification may lead to the 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 ingredients.

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

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

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APPENDICES

Susceptibility of clinical isolates against selected plant extracts exhibiting high antibacterial activity

Staphylococcus aureus clinical isolates

Plant & part extracted	solvent	Mean diameter of growth of inhibition zones, in mm																	
		1 Ab	2 Ab	3 Ea	4 Ea	5 Ea	6 Ea	7 Ea	8 Ea	9 Ea	10 Ea	11 Ea	12 Ea	13 Ea	14 Sp	15 Sp	16 Wo	17 Wo	18
1/S	MeOH	-	18	18	15	11	15	23	30	16	20	25	20	16	14	15	12	15	1
	H2O	17	15	20	20	15	20	16	20	-	16	16	20	18	17	17	18	18	1
2/LY	MeOH	22	23	20	24	20	18	22	-	20	18	20	23	20	15	18	20	18	1
	H2O	17	20	-	21	20	17	17	15	20	19	18	17	18	20	15	18	16	1
3/L	MeOH	18	18	15	-	18	16	19	15	15	-	-	21	15	30	16	15	19	2
	H2O	-	15	-	-	-	-	-	-	-	-	-	15	15	-	-	-	-	
4/L	MeOH	30	26	23	23	24	25	31	23	29	24	30	20	19	19	23	31	18	2
5/L	MeOH	30	25	21	25	30	31	30	31	26	29	25	20	23	26	25	25	27	2
	H2O	25	18	22	22	23	20	20	18	21	20	25	30	23	23	23	23	25	2
6/R	MeOH	16	-	15	20	15	19	23	20	24	33	23	20	15	33	15	34	18	1
7/B	MeOH	15	15	22	25	14	22	15	13	15	-	15	15	-	22	15	20	-	1
8/R	MeOH	15	15	20	32	17	15	12	21	15	-	20	15	-	-	15	20	-	1
9/F	MeOH	15	20	12	21	11	15	12	15	25	-	15	12	21	25	15	20	12	1
	CHCl3	17	20	15	15	15	16	13	16	19	-	15	15	15	16	15	15	12	1
	H2O	15	-	15	15	11	-	18	15	-	-	12	15	-	15	25	15	12	1
10/R	MeOH	23	15	15	15	15	15	18	14	27	13	15	15	20	-	20	25	-	
	CHCl3	15	15	15	15	-	-	15	15	15	15	15	17	15	13	15	-	-	
	H2O	25	-	-	18	-	15	15	20	22	15	-	22	30	15	-	31	-	

The number of plants refers to: 1= *Raphanus sativus,* 2= *Tamarix aphylla,* 3= *Solenostemma argel,* 4= *Mimosa pigra,* 5= *Guiera senegalensis,* 6= *Tinospora bakis,* 7= *Xeromphis nilotica,* 8= *Securidaca longepedunculata,* 9= *Kigelia Africana,* 10= *Courbonia virgata;* B= branches, F= fruit, L= leaves, Ly= leaves+young branches, R= root, S= seed; Ab= abscess, Ea= ear infection, Ey= eye infection, Sp= sputum, Wo= wound infection; (-): no inhibition

Escherichia coli clinical isolates

Plant & part extracted	Solvent	Mean diameter of growth of inhibition zones, in mm- average of 3 replicates														
		1 Ur	2 Ur	3 Ur	4 Ur	5 Ur	6 Ur	7 Ur	8 Ur	9 Ur	10 Ur	11 Ur	12 Ur	13 Ur	14 Ur	15 Ur
1/S	H2O	19	-	21	-	16	18	15	15	15	-	12	15	-	15	20
2/LY	MeOH	20	23	25	18	20	25	27	15	27	21	25	25	20	26	24
4/L	MeOH	25	28	25	19	18	28	20	18	20	19	22	27	21	28	26
5/L	MeOH	30	23	33	31	27	20	20	25	20	30	30	25	31	25	30
	H2O	18	18	20	25	22	20	18	22	20	20	19	22	23	22	20
7/B	MeOH	-	20	14	-	15	16	15	13	12	15	15	15	15	-	15
8/R	MeOH	-	-	17	-	15	15	20	12	15	-	-	18	-	-	12
9/F	MeOH	15	13	12	15	15	12	-	-	16	21	20	23	22	15	20
10/R	MeOH	17	-	-	-	15	15	15	20	-	13	22	21	15	18	19

The number of plants refers to: 1= *Raphanus sativus,* 2= *Tamarix aphylla,* 4= *Mimosa pigra,* 5= *Guiera senegalensis,* 7= *Xeromphis nilotica,* 8= *Securidaca longepedunculata,* 9= *Kigelia Africana,* 10= *Courbonia virgata;* B= branches, F= fruit, L= leaves, Ly= leaves+young branches, R= root, S= seed; Ur= urine infection; (-): no inhibition

Pseudomonas aeruginosa clinical isolates

Plant & part extracted	Solvent	Mean diameter of growth of inhibition zones, in mm-aver														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Ea	Ea	Ea	Ea	Ea	Ea	Ur	Ur	Ur	Ur	Ur	Ur	Ur	Ur	Ur
1/S	H₂O	16	-	19	17	15	-	15	15	15	13	17	15	20	15	16
2/LY	MeOH	21	19	24	22	22	23	22	25	17	21	21	20	21	25	23
4/L	MeOH	29	22	32	23	21	27	31	22	26	21	24	18	23	24	26
5/L	MeOH	23	22	21	26	26	22	21	32	26	22	25	27	17	23	25
	H₂O	20	25	21	24	22	18	20	28	27	22	20	23	20	20	22
9/F	MeOH	12	15	16	15	15	15	-	19	20	29	22	15	15	15	17
10/R	H₂O	19	15	-	13	-	12	13	-	-	26	16	15	16	19	36

Continuation of *Pseudomonas aeruginosa* clinical isolates

Plant & part extracted	Solvent	Mean diameter of growth of inhibition zones, in mm-												
		21	22	23	24	25	26	27	28	29	30	31	32	33

		Ea	Wo	Wo	Ea	Wo	Wo	Ea	Wo	Wo	Wo	Wo	Wo	Wo	
1/S	H₂O	20	-	15	20	19	21	19	-	-	23	21	19	19	
2/LY	MeOH	20	20	21	23	22	23	23	21	30	25	23	19	20	
4/L	MeOH	30	24	35	19	23	18	20	21	15	15	18	20	17	
5/L	MeOH	20	22	22	22	20	17	27	23	23	24	21	25	22	
	H₂O	20	20	22	20	15	17	18	19	20	15	20	18	15	
9/F	MeOH	12	15	15	15	15	17	15	199	15	17	-	12	17	
10/R	H₂O	15	20	22	15	41	20	25	25	15	15	23	-	25	

The number of plants refers to: 1= *Raphanus sativus,* 2= *Tamarix aphylla,* 4= *Mimosa pigra*, 5= *Guiera senegalensis,* 9= *Kigelia Africana,* 10= *Courbonia virgata;* F= fruit, L= leaves, Ly= leaves+young branches, R= root, S= seed; Ea= ear infection, Ur= urine infection, Wo= wound infection; (-): no inhibition

Proteus vulgaris clinical isolates

Plant & part extracted	Solvent	Mean diameter of growth of inhibition zones, in mm-average o														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
		Ea	Ea	Ea	Ea	Ea	Ea	Ur	Ur	Ur	Ur	Ur	Ur	Ur	Ur	Ur
1/S	H₂O	-	15	17	17	15	16	18	18	18	15	15	15	15	-	17
2/LY	MeOH	-	20	23	21	20	20	20	21	25	21	23	23	25	19	24
3/L	MeOH	-	17	38	24	18	33	46	40	34	18	31	15	18	20	20
4/L	MeOH	-	16	24	21	20	20	23	20	15	15	18	18	21	25	17
5/L	MeOH	-	23	30	17	25	23	20	30	30	25	20	27	20	23	25
	H₂O		21	25	15	23	20	18	25	25	20	18	20	20	22	25
6/R	MeOH	-	15	18	25	15	15	15	30	20	24	18	17	18	17	20
7/B	MeOH	-	15	15	-	15	-	25	35	15	13	15	13	-	15	15
9/F	CHCl₃	-	18	18	15	15	14	15	15	18	15	18	17	15	15	16

The number of plants refers to: 1= *Raphanus sativus,* 2= *Tamarix aphylla,* 3= *Solenostemma argel,* 4= *Mimosa pigra*, 5= *Guiera senegalensis,* 6= *Tinospora bakis,* 7= *Xeromphis nilotica,* 9= *Kigelia Africana;* B= branches, F= fruit, L= leaves, Ly= leaves+young branches, R= root, S= seed; Ea= ear infection, Ur= urine infection, Wo= wound infection; (-): no inhibition