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College of Science

Effect of *Suaeda aegyptiaca* extracts on some microorganisms *In vivo* and *In vitro*.

A Thesis
Submitted to the College of Science /Al-Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology.

By

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Summary

- A series of experiments were conducted to evaluate the *in vitro* antimicrobial activity of *Suaeda aegyptiaca* crude extracts against some pathogenic microorganisms as well as the therapeutical activity *in vivo* of combination of crude extracts of (alkaloids, phenols and terpens) on mice skin that previously infected with the fungus *Trichophyton rubrum*.
- Chemical analysis showed that *S. aegyptiaca* contains different active compounds: phenols, alkaloids and terpens.
- Preliminary test of the antimicrobial activity of *S. aegyptiaca* (cold water and ethanol) extracts showed that ethanolic extract had inhibitory effect on *Staphylococcus aureus, Staphylococcus. epidermidis* and *Klebseilla pneumoniae* .While, water extract affected only *Staphylococcus aureus*, Moreover, *Escherichia .coli*, *Candida albicans* and *Trichophyton rubrum* were resistance to both extracts.
- Suspected active crude compounds (phenols, terpenoids and alkaloids) were extracted from the aerial parts of *S. aegyptiaca*, and different concentrations of them were prepared (25, 50, 75 and 100) mg/ml.
- Combined inhibitory effects of extracted compounds were tested by using concentration of 100 mg/ml. The effects were varied between a synergistic, additive, or inhibitory.
- Clinical features of animal skin infected with *Trichopyton rubrum* was represented by scaly area, irregular margin with boil formation, redness and swelling and loss of hair.
- Clinical features of animals skin infected with *Trichopyton rubrum* and treated with combination of crude extracts of (phenol, terpen and alkaloids) at concentration of (100) mg/ml was represented t by the crust tend to drop-off

spontaneously , leaving white scar , usually slightly depressed along its entire length.

- After the nine days of the treatments with crude extracts of *S. aegyptiaca*, the incision was completely covered with newly formed epithelium and the hair seen in comparism with mycodin ointment, when the healing was caused slower regenerative changes, crust tending to drop-off spontaneously and the incision which covered with new area less than those covered the area treated with *Suaeda* crude extracts, indicating the efficiency of this extract as antifungal agents.

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List of Abbreviations

Abbreviations	Mean
%	Percent
°C	Degree Celsius
A	alkaloid
cm	centimeter
D.W.	Distilled Water
g	gram
HCL	Hydrochloric acid
hr	hour
min.	minute
ml	milliliter
P	phenol
rpm	round per minute
S. aegyptiaca	*suaeda aegyptiaca*
T	terpen
v/v	volume by volume
w/v	weight by volume
WHO	World Health Organization
β	Beta

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Human was used the plants as a source of remedies for many diseases since a long time, people of all continents have this old tradition (Cohen, 2002). Despite the remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Newman *et al*., 2000).

Herbal medicine is the oldest form of healthcare known to mankind. It sometimes referred to as herbalism or botanical medicine for the use of herbs in therapy or medicinal uses (Barens, 2002). One of the basic targets of herbal medicine is the interaction between different constituents occur, enhancing activity or reducing the adverse effect, such interaction may be additive or truly synergistic in that compounds interact to produce an effect greater than the sum of individual contribution of each (Williamson, 2001)

Demand for medicinal plants is increasing in developed countries due to growing recognition of natural products, having almost no side-effects, easily available at affordable prices, and sometime the only source of health care available to the poor. It was continuous investigation for new, safe, and effective antimicrobial as alternative agents to substitute with non effective ones, so natural resources especially plants were candidates for this aim (WHO, 2001).

Recently, there has been a significant increase in the use of therapeutically active compounds extracted from plants, commonly called "phytochemicals"; these phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians (Cowan, 1999).

Research on the medicinal plant had increased and different plants have been screened for their antimicrobial activity (Tomesi *et al.,* 1996). In Iraq, many studies were conducted to evaluate the probable use of some plant materials (seeds, leaves, etc.) as antimicrobials agents (Azhar, 1998).

In folk medicine, the herb *Suaeda aegyptiaca* has been used for stomach pain and for wound and skin infection (Ghazanfar, 1994). The crude terpenoid compounds that extracted from *Suaeda aegyptiaca* were used as insecticidal agent against the mosquito *Culex pipiens* (Al-Kaphagi, 2003).

The name *Suaeda* come from Arabic word “Suwaid” meaning “Black” because the plant was turning darker or blackish when dried (Torkelson, 1996).

Dermatophytes are a group of keratinophilic fungi, which invade the superficial area of the body like the skin, hair and nails (Midgley *et al*., 1997). Dermatophytes are also known as "ringworm" fungi, this name has been in use at least from the sixteenth century and was described the circular lesion produced by dermatophytes on the skin or scalp (Kwon and Bennett, 1992).

1.2 Study Objectives

- Detection and extraction of crude active compounds (alkaloids, phenols, terpens) from aerial parts of *suaeda aegyptiaca*.
- Study the antimicrobial activity of these crude extracts against some pathogenic microbes (*In vitro*).
- Applying *S. aegyptiaca* crude extracts to treat of fungoid dermatitis caused by *Trichophyton rubrum* the in experimental animals (*In vivo*).

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal Plants

Plants are the oldest friends of mankind; they not only provided food and shelter, but also, served the humanity to cure different aliments. According to the World Health Organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for health care (Calixto, 2005). Plant extracts represent a continuous effort to find new compounds with the potential to act against multi-resistant bacteria; approximately 20% of the plants found in the world have been submitted to pharmacological or biological test (Mothana and Lindequist, 2005).

The use of plant extracts with known antimicrobial properties, can be a great significance in treatments, a number of studies have been conducted in different countries to prove such efficiency (Shapoval *et al*., 1996).

Many plants have been used because of their antimicrobial character, due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances; for example, phenolic, alkaloids or terpenoids compounds (Scalbert, 1994).

Medicinal plant in Iraq can be traced back to the Sumerian period (3000-1970 B.C.) and then to the Babylonian and Assyrian period of culture and civilization about two thousand B.C.The plant that was used for the cure of diseases was not utilized at time as drug as used today but was used as magic medicine from the people of civilization (Hobhouse *et al.,* 1981). In recent years, natural resources especially plants are candidates for new, safe, and effective antimicrobials as alternative to replace those which are now ineffective due to the resistance of many pathogenic have increase (Clark, 2000). Pharmacologists, botanists, microbiologists, and natural-products

chemists are combing the phytochemicals which could be developed for treatment of infectious diseases (Cowan, 1999).

Natural plant products could also prove useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity. The global interest in the medicinal potential of plants during the last few decades is therefore quite logical (Kaushik and Dhiman, 2000).

There is an increasing interest in plant extracts as potential therapeutic agents. The use of a mixture of natural products to treat disease has a number of interesting outcomes, most notably the synergistic effects and pharmacological action of plant extracts from commonly therapeutic areas, notably anti-infective, cardiovascular and anticancer fields (Martin and Ernst, 2003).

Biological evaluation of extracts is vital to ensure safety and efficacy, these factors are importance if plant extracts to be accepted as valid medicinal agents by the healthcare community and to have a future as therapeutic agents (Simon, 2003).

No previous study in Iraq conducted on S. *aegyptiaca* extracts as antimicrobial agent for *in vivo* activity, but some studies were conducted to evaluate the antimicrobial activity of some other plants. A series of experiments in Iraq were conducted to evaluate the antibacterial activity of extracted crude terpenoids, alkaloids and phenols from Iraqi flora like *Euphorbia granulata (*Al-Zubaidi *et al*., 2005). Maoz and Neeman (1998) reported that aqueous extracts from the leaves of *Inula viscosa* was tested for their ability to inhibit *Trichophyton rubrum* and *Microsporum canis,* the aetiological agents of dermal fungal infections in humans, these extracts produced detectable antifungal activity against these dermatophytes, also these extracts were evaluated for their activity against some bacteria.

Water extract of *Ziziphus joazeiro* and *Cisalpine pyramidalis* showed a significant antifungal activity against *Trichophyton rubrum*, *Candida guilliermondii*, *Candida albicans*, *Cryptococcus neoformans* and *Fonsecaea pedrosoi*, when compared to the antifungal agent amphotericin B (Cruz *et al.*, 2007). Antibacterial and antifungal activities of the stem bark of *Kigelia africana* were carried out using agar well diffusion technique. The results revealed that the crude ethanolic extract exhibited antibacterial and antifungal activities against *Staphylococccus aureus* and *Candida albicans* (Omonkhelin, 2007).

Study by Doughari and Nuya (2008) revealed that methanolic extract of *Deterium microcarpum* possessed significant activity against some pathogenic fungi: *Trichophyton rubrum, Aspergillus niger, Penicillium digitatum, Fusarium moxysporum, Candida albicans* and *Cryptococcus neoformans* at varying concentration. Aqueous and ethanolic extract of leaves of *Chromolaena odorata* were examined for their antifungal properties by using yeasts and filamentous fungi. Extract inhibit the *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum* with a minimal inhibitory concentration range from (62.5 to 500) µg/ml for the extract (Ngono Ngane *et al*., 2006).

2.2 Plant under the study

2.2.1 Chenopodiacea:

The *Chenopodiaceae* or Goosefoot family is a large family of mostly Perennial herbs. This family includes about 100 or 700 genus and about 1500 species from herbs to trees in the world (Heywood, 1978; Al-Musawi, 1987).

The family is mostly temperate to subtropical with its center of distribution around the Mediterranean, Caspia and Red sea (Mark, 2004).

The *Chenopodiaceae* include several species of economic important; *Beta vulgaris* (sugar beets), *Spinace oleracea* (spinach), and *Chenopodium quinoa* **(**quinoa) (Trebbi and Grath, 2004).

Several species possess antibacterial and antihypertensive properties, also mentioned in folk medicine for relief of toothache and chronic rheumatic (Rizk, 1986), while others have been utilized to desalinized irrigated farmland (Zhao, 1991). Investigation of certain species of this family revealed that they contain triterpenoid, saponins, coumarins, phenolic compounds and alkaloids (Sadykov, 1978).

Most species are adapted to live in saline environments, a salt-water marsh, or a salt-desert, so this species is a halophytes (salt-living) plant (Flowers *et al*., 1986).

2.2.2 *Suaeda aegyptiaca* (Hasselq.)Zohary

The following are names and description of *S. aegyptiaca*:

Arabic names: killam, suwwaid, tarteer and mollieh.

English name: Seablite, Seepweed.

Synonyms: *Shanginia aegyptiaca* (Hasselq) Allen.

Chenopodium aegyptiacaum Hasselq (Torkelson, 1996; Schenk and Ferren, 2001). *S. aegyptiaca* is an annual herb, 60-100 cm herb, variable in shape, ascending, and sub woody at the base (Ali and El-Gady, 1989).

It is in flower from July and October, in living stage is green, under stress conditions often yellowish, leaves very succulent and fleshy, it cannot grow in the shade. (Zohary, 1976), as shown in figure1:

Figure 1: The plants of *Suaeda aegyptiaca* (catch by author)

2.2.3 Taxonomy of *Suaeda aegyptiaca* :(Rechinger, 1964)

- Domain*: Eukaryota*
- Kingdom: *Plantae*
- Subkingdom: *Viridaeplantae*
- Phylum: *Tracheophta*
- Class: *Magnoliopsida*
- Order: *Caryophylleles*
- Family: *Chenopodiaceae*
- Genus*: Suaeda*
- Species: *aegyptiaca.*

2.2.4 Biology and Ecology of *Suaeda:*

This herb is widespread in Canary Islands, Europe, Mediterranean region, Asia, Australia, northeast coast of North America, Argentina. Many of which are adapted to live in saline soil and live in salt marches or arid saline soil (Burkill, 1985).

Plants of *S. aegyptiaca* are found in saline or alkaline wetlands or, occasionally, in upland habitats (Basswtt and Crompton, 1987). The species distributed throughout Arabia in saline habitats especially on coasts and it is grow in rather different plant communities and even as weed in irrigated gardens and fields (Waisel, 1994).

2.2.5 The Importance of *Suaeda*:

S. aegyptiaca seeds have been eaten by Native Americans and some species are used as source for black and red dye (Stephen, 1990; Fisher *et al*., 1997). The plant is rich in potassium and often burnt as source for making soap and glass in Pakistan (Chughtai *et al*., 1989)

A study by Al-Lawati *et al*., (2002) on extract prepared from eight sample of plant materials were evaluated for insecticidal activity against pulse beetle, one of these plants was S. *aegyptiaca,* and the mortality of beetle treated with ethanolic extract of this plant was 65%. Insecticidal activity has been suggested for water and ethanolic extracts of. *S. aegyptiaca,* it was demonstrated that these extract caused 28% mortality for leaf miner on cucumber (*Cucumic sativus*) (Azam *et al*., 2003).

Another study by Al-Khaphagi (2003) who reported that the crude terpenoid compounds caused mortality of 82% of Culex *pipiens* eggs. Locally, this plant has great important because it is browsed by camels; people burn this plant as firewood (Ghaudhry and Arshed, 1987).

Medicinally, stems and leaves of this plant are used for tooth and gum infections, used as snuff for dizziness, headaches, hysteria, nausea, calming the nervous system and improving poor vision (Ghazanfar, 1994).

In India, this plant has been used as a leaf-poultice for opthalmia and sores as infusion (Chopra *et al*., 1986). Antibacterial activity of aqueous extract of *S. aegyptiaca* was examined against four pathogenic bacteria: *Staphylococcus aureus, Escherichia coli, Salmonella typhi and Shigella desynterial* (Al-Mawla, 2007). *S. asparagoides* Miq. has long been prescribed in traditional oriental medicine for the treatment of hypertension and hepatitis, anti-oxidative and anti-inflammatory properties of chloform and hexan extracts of *S. asparagoides* was suggested by Park *et al*., (2007).

S. fruticosa Foresk**.** Plant is used for the treatment of wounds. It is laxative, diuretic and emetic but in excess doses causes abortion and induce vomiting (Ghaudry and Arshed, 1987). Petroleum ether, methanol, butanol and aqueous crude extracts of aerial parts of *S. vermiculata* exhibited variable degrees of antimicrobial activity against *Candida albicans* and *Fusarium oxysporumo* (Abbas *et al.,* 1996).

Aqueous extract of leaves, roots and stems of *S. maritima* showing antimicrobial activity against pathogenic fungi (Kiba *et al*., 2005). Antimicrobial activity of water, acetone, ethanol extracts of *S. fruticosa* have been studied by Samia *et al.,* (2000) against *Staphylococcus aureus ,Klebsiella pneumoniae, Escherichia coli.* Fifteen species belongs to genus *Suaeda* have been analyzed for their chemical constituents and tested in different concentrations against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis (*Al-Saleh *et al.,* 1997).

Water extract of the aerial parts of *S. fruticosa* showed hypoglycemic activity in rats (Mouhsine *et al.,* 2001). Ether, acetone, ethanol, and water extracts of *S. fruticosa* were tested against three bacterial species: *Klebsiella, Staphylococcus* and *Escherichia coli* (Javed *et al*., 2000).

It was demonstrated that fourteen fatty acid and four sterols in the methanol and chloroform extracts of *S. salsa* inhibit induced ear edema in mice by corton oil, therefore anti-inflammatory activity were suggested (Chinese Traditional Patent medicinal, 2003).

2.3 Secondary Metabolites:

The secondary metabolites are variant and complex chemical compound, these compounds have been widely investigated by organic chemists because of their interesting chemical diversity(Abuharfeil *et al*., 2000).The scientist classified it according to the chemical structure of these compounds, and other classified according to the source of metabolism process (Hartmann, 1996). Secondary metabolites, generally present at 1-3% of dry plant weight, are synthesized in specialized cells at distinct developmental stages and have highly complex structures, making their extraction and purification difficult (Dave, 2003). The most common compounds are:

2.3.1: Terpenoid compounds:

Trease and Evans, (1989) reported that terpens are hydrocarbon compounds that connect essentially to a number of Isoprene unit, the common rule to this compounds are (C_5H_8) n.

Terpenoids are aromatic chemical compounds which dissolved in fats, they are found in the cytoplasm of plant cells or in special gland like the volatile oil (Harborne, 1993). Chemically, terpenoids are generally lipid-soluble, the mainly terpenoids are essential oil include the volatile oil that responsible for the odor or smell found in many plants(Stipanovic, 1983).

Terpens are active against bacteria, fungi and viruses; the mechanism of action of terpens is not fully understood but is speculated to involve membrane disruption by lipophilic compounds (Sun *et al*., 1996; Amaral *et al*., 1998). Food scientists have found the terpenoids present in essential oils in the plants to be useful in the control of *Listeria monocytogenes* (Aureli, 1992). The terpenes include the potent anticancer drug, taxol a diterpene from the Pacific Yew, and the triterpene digitalis from foxglove which is used as an effective drug for heart failure (Wink, 1999).

2.3.2. Phenolic compounds:

This group consists of thousands of diverse molecule with heterogeneous structure with common feature, having one or more phenol rings (Harborne, 1973). Most of phenolic compounds belong to flavonoids, a group of polyphenolic compounds with 15 carbon atoms (15C) based on a skeletal structure of two benzene rings joined by a linear C3 chain(C6-C3-C6 system) (Taiz and Zeiger, 2002). Flavonoid compounds are highly characteristic of plants, many of flavonoids are easily recognized as the pigments in flowers and fruits, and occur in all parts of plants (Sawain, 1998).

The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their toxicity to microorganisms, with evidence that

increased hydroxylation results in increased toxicity (Urs and Dunleavy, 1975).

Plant phenols have ability to complex with protein by hydrogen bonding when plant cell constituents come together and the membrane are destroyed during isolation procedure, the phenols rapidly complex with protein and as a result, there is often inhibition of enzyme activity in crude plants extract (Goodwin and Gillman, 1985). The well-known phytoalexin, an anticancer agent is an example of a phenolic as are flavonoids and tannins which are found in tea, fruits and have many desirable health effects (Wink, 1999).

2.3.3. Alkaloids compounds:

Alkaloids are heterocyclic nitrogen compounds; the first medically useful example of an alkaloid is morphine which isolated in 1805 from the opium poppy *Papaver somniferum* (Fessenden and Fessenden, 1982).

Alkaloids have been found to have microbiocidal effects against *Giardia* and *Entamoeba* species, also it have antidiarrhial effect probably due to their effect on transit time in small intestine (Ghoshal and lakshmi, 1996). The mechanism of action is attributed to their ability to intercalate with DNA (Phillipson and O'Neill, 1987). Alkaloids, a major class of plant-derived secondary metabolites used medicinally, and have potent pharmacological effects in animals due to their ability to rapidly penetrate cell membranes. Nicotine, a commercially important alkaloid, is the most addictive drug used by humans. Caffeine, an alkaloid from coffee, tea and chocolate is a central nervous system stimulant and mild diuretic. Over 25 alkaloids with morphine being the most abundant and most potent painkiller. Vincristine and vinblastine, important alkaloids which are strong antineoplastics used to treat

Hodgkins disease and other lymphomas (Wink, 1999).

Alkaloids are often toxic to man and many have physiological activities, hence their wide use in medicine. They usually colorless, often optically active substances most are crystalline but a few are liquids at room temperature (Harborne, 1982).

2.4 Pathogencity of Microorganisms in this Study:

2.4.1 *Staphylococcus* aureus:

Staphylococcus aureus are Gram-positive spherical cells, usually arranged in grape-like irregular clusters. Some are members of the normal flora of the skin and mucous membranes of human, others cause suppurations, abscess formation, a variety of pyogenic infection and even fatal septicemia, Staphylococci grow readily on most bacteriological media under aerobic or microaerophilic conditions (Jawetz *et al*., 1998). Hospital infection due to *S. aureus* is a worldwide phenomenon. Patients with burns and surgery are more likely to get infection with this organism (Jaypee *et al*., 1994). *S. aureus* is recovered from a variety of infections including skin lesions, such as: abscesses, wound infections, pneumonia and others (Humphery *et al*., 1997).

2.4.2 *Staphylococcus epidermidis:*

Staphylococcus epidermidis is Gram-positive spherical pathogenic bacteria that occur in microscopic clusters resembling grapes and it was common in medical device-associated, S. *epidermidis* is an inhabitant of the skin (Jawetz *et al*., 1998). *S.epidermidis* bacteria are responsible for a growing of a number of infections such as infections often start at skin wounds caused by catheters (Davis *et al*., 1990).

2.4.3 *Escherichia coli:*

Escherichia coli is a Gram-negative bacteria, motile, rods, facultative anaerobic, related to Enterobacteriaceae family. *E. coil* is a member of the

normal intestinal flora and also found as normal flora of upper respiratory and genital tracts. *E. coli* is associated with urinary tract infection, intestinal disease and some time food poisoning (Humphery *et al*., 1997)

2.4.4 *Klebseilla pneumoniae:*

Klebseilla pneumoniae is Gram-negative, non motile, encapsulated, facultative anaerobic, rod shaped. *Klebseilla* species exhibit mucoid growth, found in the normal flora of the mouth, skin and intestines, the most clinically important member of the *Klebseilla* genus of Enterobacteriacea was *Klebseilla pneumoniae* (Jawetz *et al*., 1998). *K. pneumoniae* can cause bacterial pneumonia and wound infections particularly in immunocompromised individuals (Podschun and Ullman, 1998).

2.4.5 *Candida albicans:*

Candida albicans is oval dimorphous yeast; it has been known for many centuries and has the potential to cause human diseases under specific circumstances and conditions, the actual taxonomy of *C. albicans* and related species has only been confirmed within the twentieth century (Pathak *et al*., 1999). *C. albicans* is a component of the normal skin flora and also the main cause of mucocutanous fungal diseases in humans (Hay, 1993). Superficial infections by *Candida albicans* are common in immunocompromised patients (Schaller *et al*., 2001).

2.5 Dermatophytes

During the past five decades, there has been a significant increase in studies concerning fungal infections in human and lower animals. Although fungal infections of the skin were (and still are) regarded as the most common and relatively beginning of the disease, their profile has taken a new and direct aspect reflected in pathological conditions (John *et al*., 1996)

Dermatophytosis is caused by fungi in the genera *Microsporum, Trichophyton* and *Epidermophyton*, these organisms called "Dermatophytes" are the pathogenic members of the keratinophilic (keratin digesting) soil fungi, *Microsporum* and *Trichophyton* are human and animal pathogens. E*pidermophyton* is a human pathogen (Muller *et al.,* 1989).

Dermatophytes are classified into three groups: *Trichophyton, Microsporum and Epidermophyton,* depending on the basis of clinical aspect of the disease combined with the cultural and microscopic characteristics of the fungus by developing a media containing crude pepton with either crude maltose or honey (Haward and Haward, 1983).

These types of the pathogenic fungi reproduced asexually by the formation of conidia. The conidia are asexual spores produced by specialized vegetative hyphal stalk called "conidiophores" (Weitzman and Summerbell, 1995).

Dermatophytes species can be categorized on an ecological basis as being geophilic, zoophilic or arthrophilic. The geophilic species are those whose natural habitat is the soil. Natural habitats of zoophilic dermatophytes are domestic and wild animals. The arthrophilic dermatophilic are those species that cannot survive in soil, do not infect lower animals and whose natural habitat is the keratinous tissues of human body (Kaaman, 1985; Howell *et al*., 1999).

2.5.1 Mechanism of Pathogenesis:

All dermatophytes are keratinophilic, but they differ in the kind of keratin they prefer. This amazing preference determines the site of infection of various dermatophytes (Myrvic and Weiser, 1988; Rippon, 1988).

Dermatophytes cause tinea and related diseases. The word "tinea" comes from the Latin word meaning "a growing worm", the common name

for some tinea is ringworm (Myrvic and Weiser, 1988).

In ringworm, fungi grow in a widening circle or ring that appear brownish to red as a result of the inflammatory response at this site. The center of ring is scaly, thickened infected areas of keratinized layers of the skin tend to scale off (Friedlander, 2000).

Fungal infections of skin can be classified according to their sites of infection, into the following groups:

- Superficial infection caused by fungi capable of utilizing keratin for their nutrition and invading such keratinized tissue as the stratum corneum of the skin, nails and hair.
- Cutaneous and Subcutaneous infections produced by various moulds and yeast which are usually acquired through traumatic implantation.
- Cutaneous manifestation of life-threatening visceral or systemic fungal infection in immunologically competent or immunocompromised patients caused by dimorphic fungi and other opportunistic fungal pathogens (Odds *et al*., 1992).

2.5.2 *Trichophyton rubrum*

- Pathogenesity: Infect the skin and nails, rarely infect the hair and scalp.
- Rate of growth: Slow, mature within 14 days.
- Colony morphology: surface granular, white; while the reverse of dish cultured agar is deep red or purplish; occasionally brown, yellow orange or even colorless.
- Microscopic morphology: Sepetate hyphae with lateral, tear-shaped micro-conidia (figure 2). Macro-conidia may be abundant, rare or absent; when present they are long, narrow thin –walled, with parallel sides, and have two to eight cells (Weitzman and Summerbell, 1995).

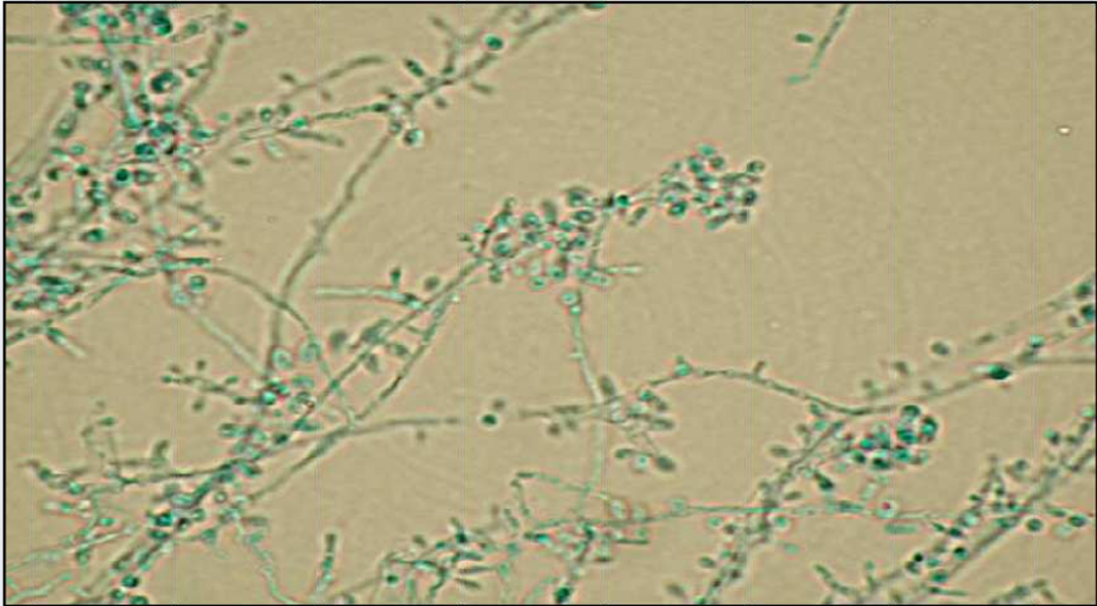


Figure 2: ***Trichophyton rubrum*** **(40x)**

2.6 Mechanisms of Resistance to Antimicrobial Drugs:

There are many different mechanisms by which microorganisms might exhibit resistance to drugs:-

- Microorganism produces enzymes that destroy the active drug.

  Example: *Staphylococcus* resistant to penicillin G produces a B-Lactamase that destroys the drug. Other β -Lactamase is produce by Gram-negative rods (Cohen *et al.,* 1988; Daley *et al.,* 1996).
- Microorganism changes their permeability to the drug.

  Example: tetracycline accumulates in susceptible bacteria but not is resistant bacteria may depend on a lack of permeability to the drugs due to an outer membrane change that impairs active transport into the cell (Livermore, 1992).
- Microorganism develops an altered metabolic pathway that by passes

the reaction inhibited by the drug (Chambers, 1999).

- Microorganism develops an altered enzyme that can still perform its metabolic function but is much less affected by the drug (Bryan, 1989; Jawetz, *et al*., 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1: Materials:

3.1.1: Table 1: Apparatus and Equipment

Equipment	Company (origin)
Autoclave	Tomy (Japan)
Distillator	GFLC (Germany)
Electrical balance	Mettler (Switzerland)
Electrical oven	Memmert (Germany)
Hot plate with magnetic stirrer	Gallen kamp (U.K.)
Incubator	Sanyo (Japan)
Laminar air flow cabinet	Gelari Class 1001 Gelman Instrument (U.K.)
Light compound microscope	Olympus (Japan)
Micropipette	Brand (Germany)
Millipore filter unit	Quick fit (U.K.)
pH-meter	Radiometer (Denmark)
Refrigerator	Ishtar (Iraq)
Rotary evaporator	Yammato (Japan)
Sensitive balance	Precisa (Switzerland)
Soxhlet	Electrothermal
Water bath	Gallenkamp

3.1.2: Chemicals

Table 2: The following chemical are used in the study:

Chemical	Supplier
Absolute ethanol	BDH (England)
Acetic acid	BDH
Agar	Oxoid (England)
Ammonium hydroxide	BDH
Bismuth subnitrate	BDH
Chloroform	BDH
Copper sulfate	BDH
D-glucose	BDH
Ethyl acetate	BDH
Ferric chloride	BDH
Hexane	BDH
Hydrochloride acid	BDH
Iodide	BDH
Lead acetate	BDH
Mercuric chloride	Fluka (Switzerland)
n-Propanol	Oxoid
Peptone	Fluka
Potassium hydroxide	BDH
Potassium iodide	BDH
Potassium iron cyanide	BDH
Roshail salt	BDH
Sodium chloride	Fluka
Sodium hydroxide	BDH
Sulfuric acid	BDH

3.1.3: Culture Media

Table 3: The following media that used in the study:

Medium	Company
Nutrient broth	Oxoid (England)
Muller- Hinton agar	Oxoid

3.1.4: Antibiotic

Table 4: **Type of antibiotic disc used in the study as a positive control:**

Antibiotic	Symbol	Concentration (µg)
Erythromycin	*E*	10
Cefataxime	*CTX*	30
Trimethoprim	*STX*	25
Gentamicin	*CN*	10
Sporalim	*SP*	25
Ampicillin	*AM*	10

3.1.5: Mycodin ointment

Mycodin ointment (an antifungal drug) was used in the study to treat the infected area, this drug used as positive control and compared with the effect of crude extracts of *Suaeda*. It's manufactures by the state company for drug industries and medical appliances, Samarra, Iraq. The mycodin ointment contains 1% Nystatin.

3.1.6: Preparation of test microorganisms:

The microorganism used for antimicrobial activity include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli, Klebseilla pneumoniae* (Bacteria), *Candida albicans*(Yeast) and *Trichophyton rubrum*(Mold). Bacteria were grown in the nutrient broth at 37°C for 24 hrs and maintained on nutrient agar slants at 4°C.While fungi *Candida albicans* and *Trichophyton rubrum* were subcultured into fresh modified Sabouraud agar slants and stored at room temperature.These microorganisms were obtained from Biology Department/College of Science/Baghdad University**.**

3.2: Methods

3.2.1: Collecting Plant Samples: -

Suaeda aegyptiaca plant samples were collected during July 2007 from Babylon University and identified kindly by Professor Dr. Ali Al-Musawi, Director of Baghdad University Herbarium, Department of Biology, College of Science, University of Baghdad. Aerial parts of this plant were cleaned with running water, and air dried at room temperature for three days, then samples were grounded into powder by electrical grinder. The powdered parts were kept in plastic bags at 4°C until use (Harborne *et al*., 1975).

3.2.2.: Experimental Animals

Healthy male BALB/C mice with age range (6-8) weeks of weight (20-30) g of strain were obtained from the house of laboratory animals at Biotechnology Research Center. Animals were kept in three small plastic cages each measured {29x12.5x11.5} cm, and contained 3 males. Floors of the cages were covered with the soft crushed wood shaving. The cages were washed with soap and tap water, and then sterilized with 70% ethyl alcohol regularly throughout the period of the study (Peter and Pearson, 1971). Animals were kept under suitable environmental conditions of (26-28°C) and 14 hour day-light program daily. Tap water and pellet were accessible freely (Vodopich and Moore, 1992).

Three groups of mice were divided as following:

- First group: control without treatment (3 mice).
- Second group: Infected with *Trichophyton rubrum* then treated with combination of (alkaloids, phenols and terprnoids) from crude extract (3 mice).
- Third group: Infected with *Trichophyton rubrum* then treated with mycodin ointment (3 mice).

3.2.3 Animals Treatment:

Hair of the skin of the mice's back was shaved, cleaned and disinfected with cotton swab saturated with 70% alcohol, scraping the skin of each mouse was done by using pathological scalpel to produce abrasion, then after half an hour, a micropipette was used to smeared 0.1ml of the spore suspensions (adjusted to 4×10^4 spore/ml) with *Trichophyton rubrum* on the abrasion .The second group was treated topically with combinations of *S. aegyptiaca* crude extract at concentration (100) mg/ml that applied on either sides of shaved surface of back after 15 days of infection when the lesions were exerted; the applied area of the skin was examined daily in comparison with control animal. While the third group was treated topically with mycodin ointment. Then the therapeutic effect of plant extract was studied and compared with that of mycodin ointment.

3.2.4: Preparation of Different Plant Extracts:-

3.2.4.1-Water Extract:

Water extract of plant was prepared by weighting 50g of plant powder in flask, and 0250ml of distilled water was added then mixed with a magnetic stirrer for two hours. The mixture was filtered through filter paper (Wattman No. 1). The supernatant was evaporated at 40ºC to dryness under reduced pressure in a rotary evaporator, then the aqueous extract left at room temperature to remove any exess water (Harborn, 1982).

3.2.4.2- Ethanolic Extract:

A quantity of 50g of plant powder was mixed with 250ml of 70% ethanol by Soxhlet apparatus for 8 hours at (40-60) ºC. The solution was evaporated to dryness in a rotary evaporator (Harborn, 1984).

3.2.4.3-Extraction of Crude Phenolic:

Twenty grams of each plants powder were extracted with 400ml of 4%HCL using reflex apparatus for one hrs. The solution was filtered through filter paper (Whattman No.1), then transferred to a separation funnel; 25ml of n-propanol was added to the residue in the separation funnel. Sodium chloride was added until the solution was separating into two layers, the upper layers, which contain phenolic compounds, was collected and evaporated until dryness by rotary evaporator at 40ºC (Ribereau Gayon, 1972).

3.2.4.4-Extraction of Crude Terpenoids:

A quantity of 50g of plant powder was mixed with 250ml of chloroform by Soxhlet apparatus for 8 hours at (40-60) ºC.The solution then evaporated to dryness in a rotary evaporator.(Harborne, 1984).

3.2.4.5-Extraction of Crude Alkaloids:

Fifty grams of plant powder was extracted with 250ml of 70% ethanol using soxhlet apparatus at (40) ºC for 24hrs, then filtered through filter paper (Whattman No.1). Ammonium hydroxide (1%) was added to make pH=9, then the solution was transferred to a rotary evaporator at (40) ºC and, transferred in a separating funnel, 10ml of chloroform was added to the residue. The chloroform layer was collected; this step was repeated for three times. The collected chloroform layers were mixed and evaporated in a rotary evaporator representing the total alkaloids (Harborne, 1973).

3.2.5: Preparation of Reagents and Solutions: -

3.2.5.1: Reagent: -

The following reagents were prepared and used for detection active compounds in the plant extract:-

3.2.5.1.1: Mayer's Reagent:

Two stock solutions were prepared:-

Solution A: thirty five of mercuric chloride was dissolved in 60ml of D.W.

Solution B: five grams of potassium iodide was dissolved in 10 ml of D.W., solutions A and B were mixed and the volume completed to 100ml with D.W. (Smolenski *et al*., 1972).

This reagent was use for detection of alkaloids.

3.2.5.1.2: Dragendorff's Reagent:

Solution A: 0.6g of bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of D.W.

Solution B: 6 g of potassium iodide was dissolved in 10 ml of D.W.. Solutions A and B were mixed together with 7 ml of concentrated hydrochloric acid and 15 ml of D.W., the whole was completed to 400 ml with D.W. (Harborne, 1973). This reagent was use for detection of alkaloids.

3.2.5.1.3: Fehling Reagent:

Two stock solutions were prepared:-

Solution A: 35 g of copper sulfate was dissolved in 100ml of D.W., then diluted by D.W. till volume 500ml.

Solution B: 7 g of sodium hydroxide and 175 g of roshail salt were dissolved in 100 ml of D.W.; the volume was completed to 500 ml of D.W. Solutions A and B were mixed in equal volumes (Sarkas *et al*., 1980).This reagent was use for glycosides detection.

3.2.5.2: Solutions:

The following solutions were prepared and used for the detection of active compounds in plant extracts:

3.2.5.2 .1: Lead acetate solution 1 %(w/v): Prepared by dissolving 1 g of lead acetate in 100 ml D.W., it is used for tannins detection.

3.2.5.2.2: Ferric chloride solution 1%(w/v): Prepared by dissolving 1 g of ferric chloride in 100 ml D.W.. It is used for tannins and general phenols detections.

3.2.5.2.3: Potassium hydroxide solution 50%(w/v): Prepared by dissolving 50 g of potassium hydroxide in 100 ml of D.W.. This solution is used for the detection of flavonoids.

3.2.5.2.4: Mercury chloride solution 1 %(w/v): Prepared by dissolving I g of mercuric chloride in 100 ml of D.W.. It is used for saponin detections.

3.2.6 Detection of Active Compounds

3.2.6.1- Detection of General Phenols:

Two ml of aqueous ferric chloride 1% was mixed with two ml potassium iron cyanide 1%. Equal quantity of reagent (3.2.5.2.2) and aqueous plant extract were used. The appearance of blue-green color indicated the presence of phenols (Harborne, 1984).

3.2.6.2- Detection of Tannins:

Ten grams of the plant powder was mixed with 50 ml of D.W. by using a magnetic stirrer, the mixture was left in a boiling water bath for few minutes, then filtered it, the supernatant solution was separately treated with the two following aqueous solutions according to the following steps:

A) Aqueous lead acetate 1% solution, the gelated precipitate was an indication of the presence of tannins (Al-Muktar, 1994).

B) Aqueous ferric chloride 1% solution, the development of greenish-blue precipitate is an indication for the presence of tannins (Harborne, 1982).

3.2.6.3- Detection of Flavonoids:

A) Ten grams of powder plant were dissolved in 5 ml ethanol 95% then filtered it,

B) Ten ml of ethanol 50% were added to 10 ml of aqueous potassium hydroxide 50%, solution **A** was mixed with solution **B,** the appearance of yellow color evidence of the presence of flavonoids (Harborne, 1984).

3.2.6.4- Detection of Saponins:

A) Foam test for aqueous extract: Thick foam resulted from shaking an indication for presence of saponin.

B) Aqueous mercury chloride 1%: Five milliliters of aqueous plant extract was added to (1-4 ml) of aqueous mercury chloride 1%, presence of white precipitate indicated of saponin presence (Al-Mukter, 1994)

3.2.6.5- Detection of Glycosides before hydrolysis:

Equal amounts of the water extract was mixed with Fehling's reagent in a test tube, then boiled in a water bath for 10 minutes. Formation of red precipitate indication for the presence of glycon part of glycosides (Stahl, 1969).

3.2.6.6- Detection of Alkaloids:

Ten grams of plant powder was boiled with 50 ml of distilled water and 2ml of 4% HCL, then filtered. Half milliliter of this solution was added to 0.5 ml of each following indicators:-

A) Dragendroff's Reagent:

After added 1-2 ml of the reagent, appearance of orange precipitate indicated the presence of alkaloids (Harborne, 1973).

B) Mayer’s Reagent:

After added 1-2 ml of the the reagent, appearance of white precipitate indicated the presence of alkaloids (Smolensk *et al.,* 1972).

C) Tannic acid Reagent:

One gram of tannic acid was added to 100ml of distalled water and when used 1 ml of reagent was added to 1-2 ml of aqueous or alcoholic extract. Appearance of white precipitate indicated the presence of alkaloids (Harborne, 1973).

3.2.6.7- Detection of Terpens:

One gram of ethanolic plant extract was precipitate by a few drops of chloroform and then 3 drops of acetic anhydride and 2-3ml of concentrated sulfuric acid were added. Appearance of dark brown color indicating of the presence of terpens (Al-Abid, 1985).

3.2.6.8- Detection of Resins:

Fifty ml of ethanol (95%) were added to 5 g of plant powder and boiled in water bath for two min. The solution was filtered and then 100ml of D.W. (with 1-2 ml of 4%HCL) was added. Appearance of turbidity indicated the presence of resins (Shihata, 1951).

3.2.7: Measurement of the Extract Acidity:

Ten grams of *S. aegyptiaca* powder were mixed with 250ml of D.W. for 10 min by magnetic stirrer. The suspension was filtered and the acidity of the filtrate was measured by using pH meter (Shihata, 1951).

3.2.8: Sterilization Methods

A-Culture media were sterilized by autoclaving at 121ºC/15 min (15 lb/ $inch^2$)

B-Glasswares were washed with sterile distil water several times then, sterilized by electric oven (180-200ºC) for 3-2 hr. **(**Cappuecino and Shermany, 1987).

C- Plant extracts:

The prepared stock solutions of *S. aegyptiaca* extracts were sterilized by using Millipore filter unit with pore size of 0.22µ. (Adeeba, 1982).

3.2.9: Preparation of Modified Sabouraud Agar:

Trichophyton rubrum was cultured on modified Sabouraud dextrose agar prepared according to Finegold *et al*., (1982) by mixing the following ingredients:

Peptone	10 g
Glucose	20 g
Agar	20 g
Cycloheximide	0.5 g
Cephalexin	0.5 g
Distilled Water	1000 ml

The cycloheximide was added to this media after sterilization to prevent the growth of saprophytic fungi, while Cephalexin was added to prevent the growth of bacteria (Beneke and Rogers, 1980).

3.2.10: Preparation of Bacterial Culture Media: -

The bacteria were cultured on nutrient agar by mixing 20 g of agar with 8 g of the ready to use nutrient broth powder in a conical flask, and then the volume was completed to (1) liter with distilled water. (Tilton *et al*., 1992).

3.2.11: Spore Suspension

Spore suspension were prepared according to Faraj method (Faraj, 1990), spore were harvested by adding 5 ml of sterilized distilled water

containing 0.1% tween 80 to aid wetting and separation of the spores, then the fungal growth was separated by a loop. The suspension was filtered through sterile cotton wool, then filtrate was centrifuged at (300) rpm, further washed with distillated water. The suspension was removed and the spores were washed twice by resuspending in sterile distilled water and further centrifuged. Then 5 ml of sterile distilled water was added to the supernatant and mixed vigorously by vortex for 1 min. One drop of the supernatant was added to haemocytometer by Pasture pipette, spores were calculated under high power (X40) of light compound microscope using the following equation:

Concentration of spores= $Zx4x10^6$/ n (spores/ ml)

Where n: total n. of small squares

Z: total n. of spores.

3.2.12: Preparation of Extracted Antimicrobial Compounds: -

The stock solutions of alkaloids and phenolic extracts to be used as antimicrobial agent were prepared by dissolving (3) g of each plant extract residue with (5) ml 70% ethanol then completed the volume to 30 ml with D.W., while, the stock solution of terpenoid prepared by dissolving (3) g of plant extract residue in (2.5) ml of 70% ethanol and (2.5) ml chloroform then the volume completed to 30 ml with D.W.. Different concentrations (100, 75, 50, and 25mg/ml) of the plant extracts were prepared according to this equation:

$$C_1V_1=C_2V_2$$

Agar-well Diffusion and paper discs tests were used for testing the effect of plant extracts on pathogenic microorganisms

While antifungal samples were prepared by adding plant extracts at different concentration (100, 75, 50, and 25mg /ml) to the modified

Sabouraud dextrose agar. All petridishes were inoculated with fungus spore (adjusted to 4×10^4 spore/ml) and then incubated at 37C° for 7-10 days (Al-Samarei *et al.,* 2001). The diameter of fungal colonies was determined after the periods of incubation, then the inhibition percentage were calculated according to the following equation:

$$\text{Inhibition percentage} = \frac{\begin{matrix}\textbf{Average of diameter of fungal growth in} \\ \textbf{control plate}\end{matrix} - \begin{matrix}\textbf{Average ofdiameter of fungal growth in} \\ \textbf{treated plate}\end{matrix}}{\textbf{Average of diameter of fungal growth in control plate}} x100$$

3.2.13: Antibacterial Susceptibility Testing

3.2.13.1 Agar-well Diffusion Method

The crude extracts of phytochemicals (alkaloid, phenol and terpen) were tested on the agar-well diffusion test as described by Saxena *et al.,* (1995), 0.2 ml of 24 hr bacterial culture (1.5×10^8 cell/ml) was introduced spreaded by a sterile glass rod on the surface of solidify Muller-Hinton agar plates. Five wells of about 0.6 mm diameter were punched on agar plate using a sterile cork borer allowing at least 30 mm between adjacent wells and peripheral wells and the edges of the Petri dish. Fixed volumes (0.1 ml) of the crude extract at the concentrations (25, 50, 75 and 100) mg/ml were then introduced into wells in the plates. A control well was in the center of the plates with 0.01 ml of the extracting solvent diluted with distilled water. The inoculated plates were incubated at 37ºC for 24 hr. Duplicate plates were used in all the experiments.

3.2.13.2: Paper Discs Diffusion Method

Sterile paper discs(6 mm, Whatman No. 1) were soaked in different concentrations of the crude extracts(25, 50, 75 and 100 mg/ml) for 2h, 0.2 ml

of 24hr bacterial culture($1.5x10^8$ cell/ml) of the bacteria was spreaded on the surface of solidify Muller-Hinton agar plates. Paper discs containing the extracts were placed at different areas on the surface of each plate. The plate were incubated at 37ºC for 24 hr. Antibacterial activity of the extract against the test bacteria was indicated by growth-free "zone of inhibition" near the individual disc(Addy *et al.,* 1989).

3.2.14: Statistical analysis

Statistical analysis tests were used depending on the nature of data. The standard statistical methods were used to determine the mean and standard error of mean, one way analysis (ANOVA) using programs (SPSS) to report the level of statistical significance among the mean of different group. In all cases means are shown with ± standard error, and (T-test) program was used to determine the significant variations between groups at $p \leq 0.05$. (Soft ware program)(Box, *et al*., 1978).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1: Plant Extract:

4.1.1: Water Extract

The weight of the dry material resulted from evaporation of water was 4.3 g, it appeared with brown color. This sample contains all ingredients that were soluble in water such as gums, and resins (Al-Rawi and Chakravarty, 1988).

4.1.2: Ethanolic Extract:

The weight of the dry material resulted from evaporation of ethanol was 3.7 g, it appeared with brown color.

4.1.3: Extraction of Crude Terpenoid Compounds

The weight of dry material resulted from evaporation of chloroform was 2.5 g with green color. Terpenoids are generally lipid-soluble and located in the cytoplasm of the plant cell and normally extracted with from plant tissue with chloroform (Sun *et al*., 1996).

4.1.4: Extraction of Crude Phenolic Compounds

The weight of residue obtained was 4.5 g with dark green color.

4.1.5: Extraction of Crude Alkaloids Compounds

The resulted weight of the ethanolic extract is 5.6 g after evaporation of ethanol and appeared with dark green color (Table 5).

The relative proportion between the amounts of plant used for extraction and crude products was variable depending on several factors, such as the methods of extraction and solvent used in extraction process, as well as, the plant species (Henning *et al*., 2003).

Table 5: Different plant extracts were used

Plant Extract	Dry weight (g)	Remarks
Water extract	4.3	brown color
Ethanolic extract	3.7	brown color
Crude phenolic extract	4.5	dark green
Crude alkaloidal extract	5.6	dark green
Crude terpenoid extract	2.5	green

4.2: Detection of Active Compounds:

After using different chemical reagents and solutions, various active compounds were detected in *S. aegyptiaca* as shown in (Table 6). Result obtained by chemical detection indicated the presence of phenols, flavonoids, saponines, tannins, glycosides, alkaloids, terpens and resins in *S. aegyptiaca* extract. Such finding are in agreement with the results of Al-kaphagi, (2003) who reported that aqueous extract of S. *aegyptiaca* contain alkaloids, phenols, terpens, saponins, flavonoids, tannins. The pH of water extract of *S. aegyptiaca* was 6.6 which represent the weak acidity.

This property for the water extract of *S. aegyptiaca* may enable the plant to resist the microorganisms. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products are present in the plant. The medicinal actions of plants are unique to particular plant species or groups are consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to the primary products, such as carbohydrates, lipids, proteins, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman *et al*., 1999; Wink, 1999).

Secondary metabolites of plants play an important role in the scientific research and the aspect of drug production, due to the availability of plants all around the world and their activity in many therapeutic sides (Uglyanista *et al*., 2000).

Table 6: Active compounds detected in *S. aegyptiaca* plant extracts (all were positive).

Compounds	Reagents	Type of extract	Result obtained
- pH	pH meter	Water	6.6
-General phenols	aqueous ferric chloride+potassium iron cyanide	Water	Blue-green color
-Tannins	Aqueous lead acetate Aqueous ferric chloride	Water Water	Gelated precipitate Blue-green color precipitate
-Flavonoids	Ethanol+KOH	Ethanol	Yellow color
-Saponins	Foam test Aqueous mercury chloride	Water Water	Thick foam White precipitate
-Glycosides	Fehling´s reagent	Water	Red precipitate
-Alkaloids	-Mayer´s reagent -Dragendorff´s reagent	Water Water	-White precipitate -Orange precipitate
-Resin	Ethanol+ boiling DW	Ethanol	Turbidity
-Terpens	Chloroform+sulfuric acid+acetic anhydried	Ethanol	Dark brown color

4.3: Susceptibility of Microorganism to for Antibiotics

Results shown in (Table 7) indicated that gram-positive bacteria (*S. aureus* and *S. epidermidis*) were more sensitive to antibiotic as compared with the gram-negative bacteria;(*E. coli* and *K. pneumoniae*).While *Candida albican* was sensitive to Sporalim drug. Another notice, that *S. aureus* was resistance to Ampicillin which is derivative of Penicillin, because the production of β–Lactamase (Goodman and Gillman, 1985; Hugo and Russel, 1981).

The resistance of tested bacteria may be attributed to the following (Gilland and Murray, 1988):

- Cell membrane permeability.
- Genetic mutation.
- Creation of another means of resistance to antibiotics.

The conventional antibiotic cefataxime, showed superior activity than water and ethanolic extracts. This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of manufacturing techniques and procedures, herbal medicinal products are prepared from plant and animal origins, most of the time subjected to contamination and deterioration. In addition that, the storage of extracts like any other pharmaceuticals requires special conditions of humidity, temperature and light (De and Ifeoma, 2002).

Table 7: Susceptibility of test bacteria and yeast to six antibiotics:

Microorganism / Antibiotic	*S .aureus*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*C. albicans*
CTX	S	S	S	S	/
AMP	R	R	S	S	/
E	R	R	R	R	/
SP	/	/	/	/	**S**
SXT	S	S	R	R	/
CN	S	S	R	R	/

S: Sensitive

R: Resistance

/: Not used

4.4: Preliminary activity of *S. aegyptiaca* extracts.

Activity of *S. aegyptiaca* crude extract on some pathogenic microorganisms was conducted. Although both water and ethanolic extracts of the aerial parts *S. aegyptiaca* have antimicrobial action on the tested microorganisms, ethanolic extract was more effective than water extract (Table 8). Ethanolic extract showed antimicrobial effect on *S.aureus, S.epidermidis* and *K. pneumoniae*. While, water extract affected only *S.aureus*. On the other hand, *E. coli*, *C. albicans* and *T. rubrum* were resistance to both crude extracts; this tends to show that the active ingredients were better extracted with ethanol than water. Akunyili *et al*., (1991) observed the closed results when they worked with the stem bark of *Kigelia pinnata*. The resistance of fungi to water extracts may attributed to the presence of carbohydrate, proteins and organic materials which could be found in water extract, in general these type of active ingredients encourage the growth of fungus (Khan *et al*., 1993).

The effect of ethanolic extracts may be due to the presence of alkaloids and phenols (Azhar, 1998). The ethanolic extract of some medicinal plant such as *Carica papaya* showed significant activity on pathogenic bacteria such as: *Staph. aureus, Bacillus cereus, E.coli, Pseudomonas aeroginosa* and *Shigella flexneri* (Emeruwa, 1992). Another notice in present study, that the agar well diffusion and papers disc diffusion tests proved to be good, but the results was showed the agar well diffusion test showed wider zones of inhibition than papers disc diffusion test.This probably because the paper disc may retains the active component and does not allow it to diffuse into the Muller Hinton Agar as mentioned by Essawi and Srour (2000) who found that the agar well diffusion method were more preferred over disc diffusion one.

Table 8: Activity of Water and Ethanolic extracts of *S. aegyptiaca* on tested microorganisms.

Microorganism / Type of extract	*S. aureus*	*S.epidermidis*	*E. coli*	*K. pneumoniae*	*C.albicans*	*T. rubrum*
Water extract	8-12	≤ 6	-	-	-	-
Ethanolic extract	13-15	8-12	-	8-12	≤ 6	-

4.5: Effect of Plant Extracts on Growth of pathogenic microorganisms.

The crude extracts of Phenol, alkaloid and terpen were prepared from aerial parts of *S. aegyptiaca* with different concentrations started with (25, 50, 75and100) mg/ml. The antimicrobial activity of S. *aegyptiaca* extracts is only detectable at very high concentration of the extract (up to 50 mg/ml of crude extract). Generally the antimicrobial activity of any extract depends on the type of extract, concentration and the type of microorganisms (Rauha *et al.,* 2000). The active compounds that obtained from this plant showed varying levels of activity against all tested microorganisms. This suggests that it has a broad spectrum of activity although the degree of susceptibility could differ between different microorganisms.

4.5.1: Effect of crude phenolics extract of S. *aegyptiaca* on some pathogenic microorganism.

Results indicated that crude phenolic extract had inhibitory activity against the tested microorganisms in different concentrations. Crude phenolic extract of *S. aegyptiaca* showed significant inhibition zones to *S .epidermidis* in all concentrations (25, 50, 75,100) mg/ml, compared with inhibitory effect of extracts on *S. aureus* , *E .coli*, and *C.albicans* that inhibited at (50, 75 and 100) mg/ml, but there was no significant inhibition at 25 mg/ml, while, *K. pneumoniae* was inhibited only at (75 and 100)mg/ml, as shown in (Table 9 and figure 3). Result also showed that the sensitivity of above microorganism to the phenolic compounds was more than of the effect of some antibiotics used against these microorganisms. Variations in the susceptibility of bacterial species may be due to genetic variations, cellular structure and the target, which affected by these compounds (Mitscher *et al*., 1972; Frazier and Westhoff, 1987). Furthermore present results disagreed with those Al-Oubaidi (2003) who mentioned that crude phenolic extract of *Datura metel* showed strong inhibition zones against gram- positive bacteria that caused otitus media in all concentrations used.

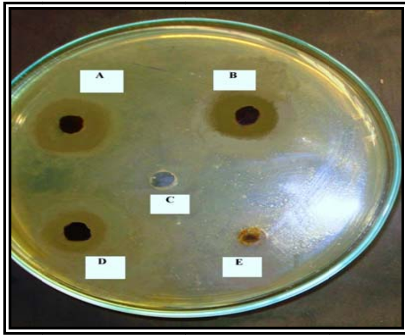
A direct correlation between extract concentration and inhibition zone diameters was found. Hernandez *et al*., (1994) found the same correlation between the concentration of methanolic extract of *Visnea mocanera* leaves and the inhibition zone against some pathogenic bacteria. Jawetz *et al*., (1998) described the mechanism thought to be responsible for phenolics toxicity against microorganism to one or all the followings:

- Membrane disruption.
- Bind to adhesions, complex with cell wall, inactive the enzyme.
- Bind to protein.

Table 9: Effects of different concentrations of crude Phenolic compounds extracted from S. ***aegyptiaca*** **aerial parts on tested microorganisms.**

Phenolic Concentrations (mg/ml) / Microorganism	Diameter of Inhibition Zone (mm)± standard error			
	25	**50**	**75**	100
S .aureus	0.0a	12.88±1.67a	13.17±0.44a	14±1.15a
S. epidermidis	10.5±0.5b	11.0±2.68b	12.5±1.45a	17.5±0.5b
E. coli	0.0a	12 .5±2.5c	14.0± 1.7b	16.5±5.0c
K. pneumoniae	0.0a	0.0a	13.33±1.45c	16.0±2.0d
C.albicans	0.0a	11.0±2.68d	10.5±0.5b	11.0 ±2.9b

Different letters in the same column significant differs ($p \leq 0.05$) between means.



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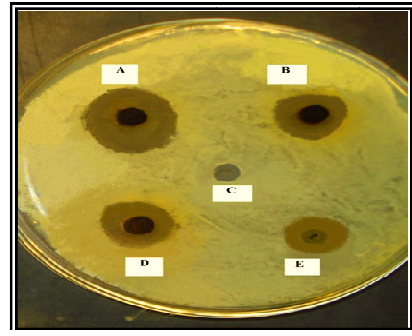

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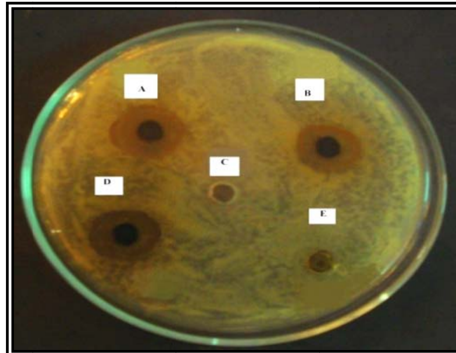

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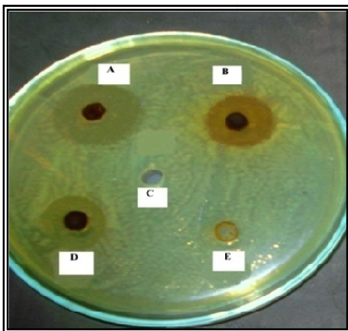

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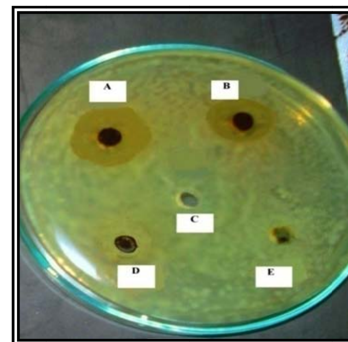

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Figure 3: Effect of crude phenolic compounds on growth of some microorganisms

1= *S. aureus*, 2= *S. epidermidis*, 3= *C. albicans*, 4=*E. coli*, 5=*K. pneumoniae*

A=100mg/ml, B=75mg/ml, C= control, D=50 mg/ml, E=25mg/ml.

4.5.2: Effect of crude alkaloids extract of S. *aegyptiaca* on pathogenic microorganism.

There was significant inhibitory effect on *S. aureus* at the concentrations (100,75,50)mg/ml, but not at 25mg/ml. *E.coli* and *K. pneumoniae* were found to be resistant to the extracted alkaloids at all concentrations used. While same extract showed significant inhibitory effect on *C. albicans* and S. *epidermidis* at concentrations 100mg/ml and 75mg/ml. (Table 10 and Figure 4). It is possible that this extract may achieve effect via an immunopharmacological mechanism. This, however, requires further investigation. Inactivity of alkaloids that extracted from some plants on some microorganisms may be due to the presence of inactive bases in its structure (Al-Shamma *et al.,* 1979).

The effect of alkaloids on microorganism may be attributed to:

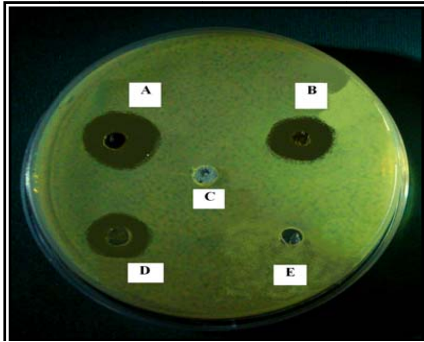
- Plasma membrane destruction.
- The interact with metabolic reactions.
- Changes in the enzymes that is vital to growth and reproductions. (Ashoor, 1988; Abdul- Rahman, 1995).

These results disagree with those Al-Ani (2006) who found that even low concentrations of the alkaloids extract of *Fumaria officinals* has antimicrobial activity, while agree with Al-Shamma *et al.,* (1979) mentioned that the alkaloids are no effect in low concentration, and agree also with the results of Al-Oubaidi (2003) who found that alkaloid has low inhibitory effect on some pathogenic bacteria at high concentration ranging (50-200) mg/ml.

Table 10: The effects of different concentrations of crude Alkaloidal compounds extracted from *S. aegyptiaca* aerial parts on tested microorganisms.

Alkaloids Concentrations (mg/ml) / Microorganism	Diameters of Inhibition Zone (mm)±standard error			
	25	**50**	**75**	**100**
S .aureus	0.0a	11.0±2.68a	13.5±2.5a	15.6±1.44a
S. epidermidis	0.0a	0.0b	16.0±0.5a	17.0±0.71b
E.coli	0.0a	0.0b	0.0b	0.0c
K.pneumoniae	0.0a	0.0b	0.0b	0.0c
C.albicans	0.0a	0.0b	10.5±0.7b	14.0 ±2.0d

Different letters in the same column significant differs ($p \leq 0.05$) between means.



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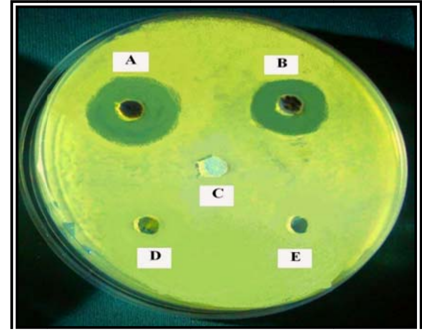

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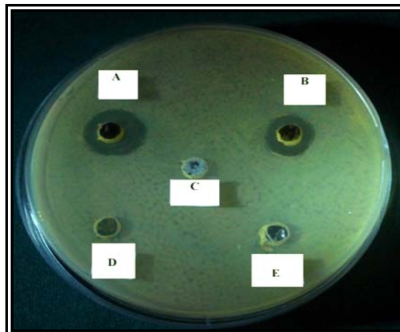

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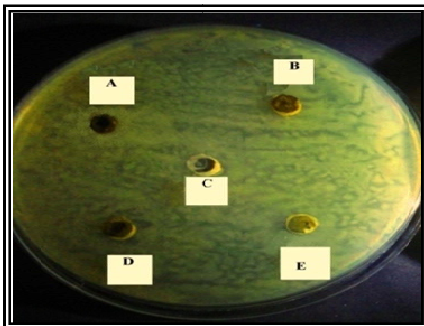

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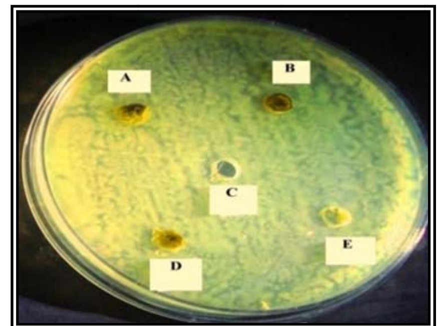

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Figure 4: Effect of crude alkaloidal compounds on growth of some microorganisms

1= ***S. aureus*****, 2=** ***S. epidermidis*****, 3=** ***C. albicans*****, 4=*****E. coli*****, 5=*****K. pneumoniae***

A=100mg/ml, B=75mg/ml, C= control, D=50 mg/ml, E=25mg/ml.

4.5.3: Effect of crude terpenoids extract of S. *aegyptiaca* on pathogenic microorganism.

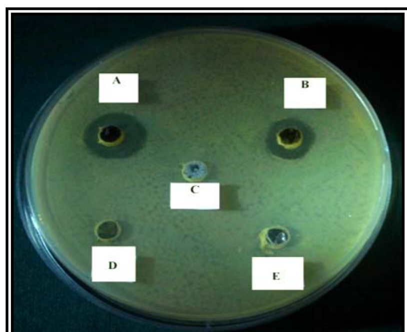
Results showed that terpenoids extracts of *S. aegyptiaca* strongly affected the growth of *S .epidermidis* at all concentrations. However, *S. aureus* and *K. pneumoniae* were significant inhibited at concentrations of 100mg/ml, 75mg/ml and 50mg/ml, while *E. coli* and *C. albicans* at 100mg/ml and 75mg/ml, (Table11 and Figure 5). Hufford *et al*., (1993) found that terpenoids showed an excellent activity against *S .aureus* and less activity against gram- negative bacteria.

Two diterpenes isolated by Batista *et al*., (1994) were found to be more selective, when they worked well against *S. aureus* and *P. aeruginosa*. The mechanism of terpens action is not fully understood, but it is speculated that it involves membrane disruption by the lipophilic compounds, Mendoza *et al.,* (1997) found that increasing the hydrophilicity of kaurene diterpenoids by addition of methyl group drastically reduced their antimicrobial activity. Same findings were observed by Taylor *et al.,* (1996) and Hernandez *et al.,* (1994).

Table 11: Effects of different concentrations of crude Terpenoid compounds extracted from *S. aegyptiaca* aerial parts on tested microorganisms.

Terpenoids Concentrations (mg/ml) / Microorganism	Diameters of Inhibition Zone (mm) ±standard error			
	25	**50**	**75**	100
Staph. aureus	0.0a	12.5±0.93a	13.5±2.5a	14.0±2.12a
Staph. epidermidis	10±2.11a	11±2.11b	13.0±1.7b	17.5±2.0a
E. coli	0.0a	0.0c	12.4±0.93c	15.6±1.44b
K. pneumoniae	0.0a	12.75±2.05d	13.5±2.7d	16.0±2.12c
C. albicans	0.0a	0.0c	11.0±0.5e	11±0.71d

Different letters in the same column significant differs ($p \leq 0.05$) between means.



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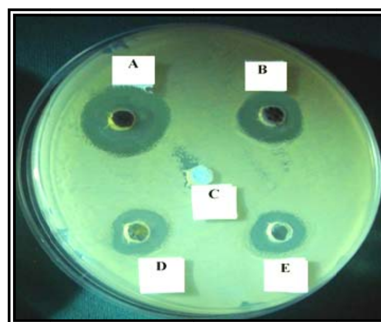

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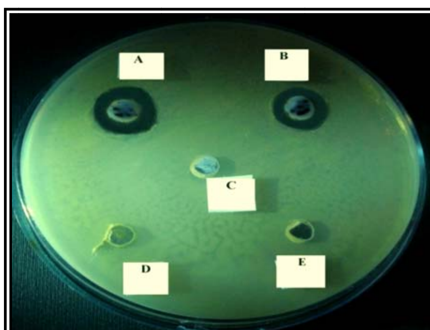

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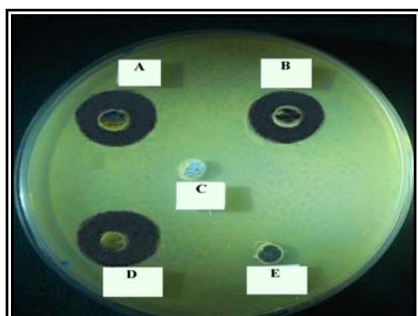

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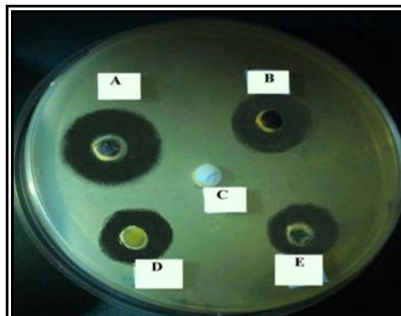

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Figure 5: Effect of crude terpenoid compounds on growth of some microorganisms

1= *S. aureus*, 2= *S. epidermidis*, 3= *C. albicans*, 4=*E. coli*, 5=*K. pneumoniae*

A=100mg/ml, B=75mg/ml, C= control, D=50 mg/ml, E=25mg/ml.

4.5.4: Combined Effect of Crude (Alkaloids, phenols and terpenoids) extracts of S. *aegyptiaca* on pathogenic microorganism.

In this section a series of experiments were conducted to evaluate the combined effect of crude extracted phytochemicals (Alkaloids, phenols and terpenoids) from *S. aegyptiaca* aerial parts on some pathogenic microorganisms. Effect of the combination of three groups of phytochemicals was varied. In the case of combination of three phytochemics: alkaloids, phenols and terpenoids (A+P+T), significant inhibitory actions were detected as compared with other combinations as shown in (Table 12). While the combinations of alkaloids and terpenoids (A+T) had no significant inhibition on tested microorganisms Another notice can be observed from (Table 12 and Figure 6), that the inhibition zone was less than that resulted from the effect of single compounds (Tables 9, 10, 11). This suggests the presence of synergistic effects, or additive effects or inhibitory effects of these compounds together.

Richard *et al*., (1991) (1995) and (1997), found that the synergistic mechanism between two or more of antimicrobial compounds or antibiotics, may lead to increase the penetration of these compounds or antibiotics to the cell membrane. Pezzuto *et al.,* (1991) in their study on the effects of Camptothecin, Bleomycin on some bacteria found that the combined effects of these antibiotics were reduced as compared with their effects separately. This concludes that this may be due to the presence of other compounds which affect the inhibitory ability when they combined.

Similar result was obtained by Al-Obaidi (2003) who reported that the combinations of (A+P+T) extracts of *D. metel* leaves showed higher effects against some bacteria agents causing otitus media.

In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process.

As pointed out by Tyler (1999), this synergistic or additive pharmacological effect can be beneficial by eliminating side effects associated with the predominance of a single xenobiotic compound in the body. Another notice that the most microorganisms that was sensitive to all the extracts was that S. *aureus* ,Similar conclusion were recorded by some other workers such as: Okemo *et al*., (2001); Madamombe and Afolayan,(2003).

In general, depending on the site of action pharmaceuticals studies of antimicrobial classified into:

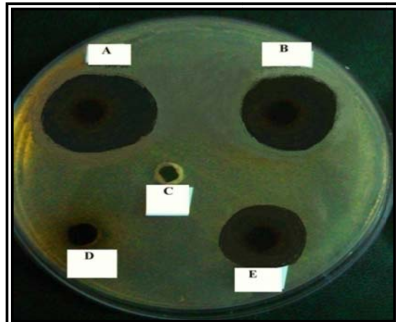
- Drug that inhibit cell wall synthesis.
- Drug that inhibit nucleic acid synthesis.
- Drug that inhibit protein synthesis.
- Drug that affecting cytoplasm membrane.

(Laurence *et al*., 1999)

Table 12: Combined effects of crude phytochemicals (terpenoids, alkaloids and phenolics) extracted from *S. aegyptiaca* aerial parts at (100 mg/ml) on tested microorganisms.

Active compounds at (100 mg/ml) concentration / Microorganism	Diameters of Inhibition Zone(mm)±standard error			
	P+T	**P+A**	**A+T**	P+T+A
S. aureus	15.6±1.44a	20.0±2.1a	0.0a	22.0±2.9a
S. epidermidis	14.0±2.12b	12.88±1.66a	0.0a	11. 0 ± 0.5b
E. coli	10.0±0.5c	16.6±1.51b	0.0a	13.5±2.5b
K. pneumoniae	12.0±1.4d	13.0±1.47b	0.0a	13.0±2.5c
C. albicans	11.0±2.68e	17.0±0.5b	0.0a	14.0±3.5d

Different letters in the same column significant differs ($p \leq 0.05$) between means.



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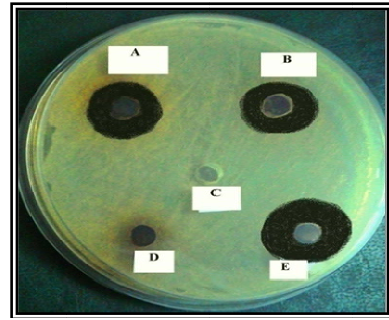

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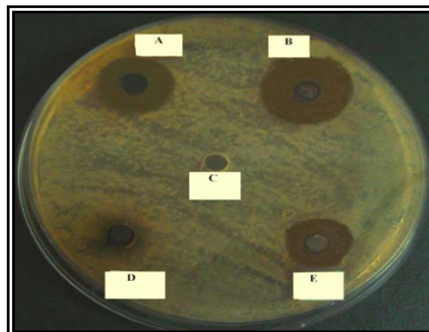

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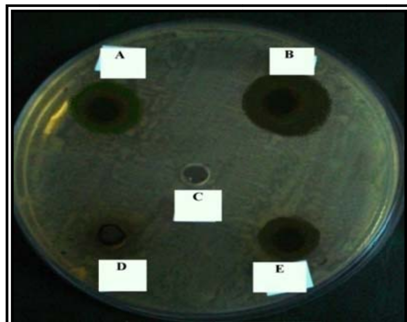

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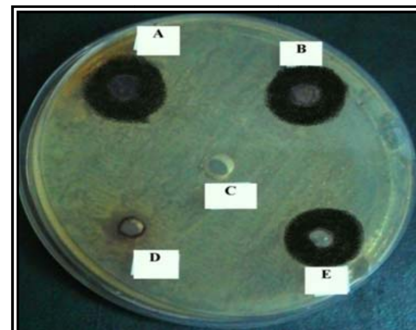

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Figure 6: Effect of different combinations of *S. aegyptiaca* crude extracts (phenol, alkaloid, terpen) at (100 mg/ml) on growth of some microorganisms

1= *S. aureus*, 2= *S. epidermidis*, 3=*C. albicans*, 4=*E. coli,* 5=*K. pneumoniae*

A=A+T+P, B=P+A, C= control, D=A+T, E=P+T

4.5.5: Effect of crude phenolic extract of S. *aegyptiaca* on *Trichophyton rubrum*.

The crude phenolic extract of *S. aegyptiaca* showed different inhibition activities against *T. rubrum*. Growth of *T. rubrum* showed significant inhibition effect at all concentrations except (100 mg/ml). However, best concentration of crude phenolic extract to inhibit growth of *T. rubrum* was 100 mg/ml, as shown in (Table 13 and Figure 7). Adedayo *et al*., (1999) demonstrated that, the methanolic extract of *Seena ataa* was active against the growth of *Aspergillus niger, Pencillium spp, Geotrichim utilis* at a minimum fungicidal concentration which was generally more than 5 mg/ml, the differences in the activity of this extract may be due to the type of flavonoids that was found in the extract.

Table 13: The Effect of Crude phenolic extract of S. *aegyptiaca* on *Trichophyton rubrum*

Concentration (mg/ml)	Average of colonial diameter (mm)±SE	Percentage of inhibition (%)
0	90.0±0.31a	0
25	72±0.76a	20
50	63.0±0.76a	30
75	52. 3 ± 0.76b	40
100	43.6±0.76c	51.11

Different letters in the same column significant differs ($p \le 0.05$) between means.

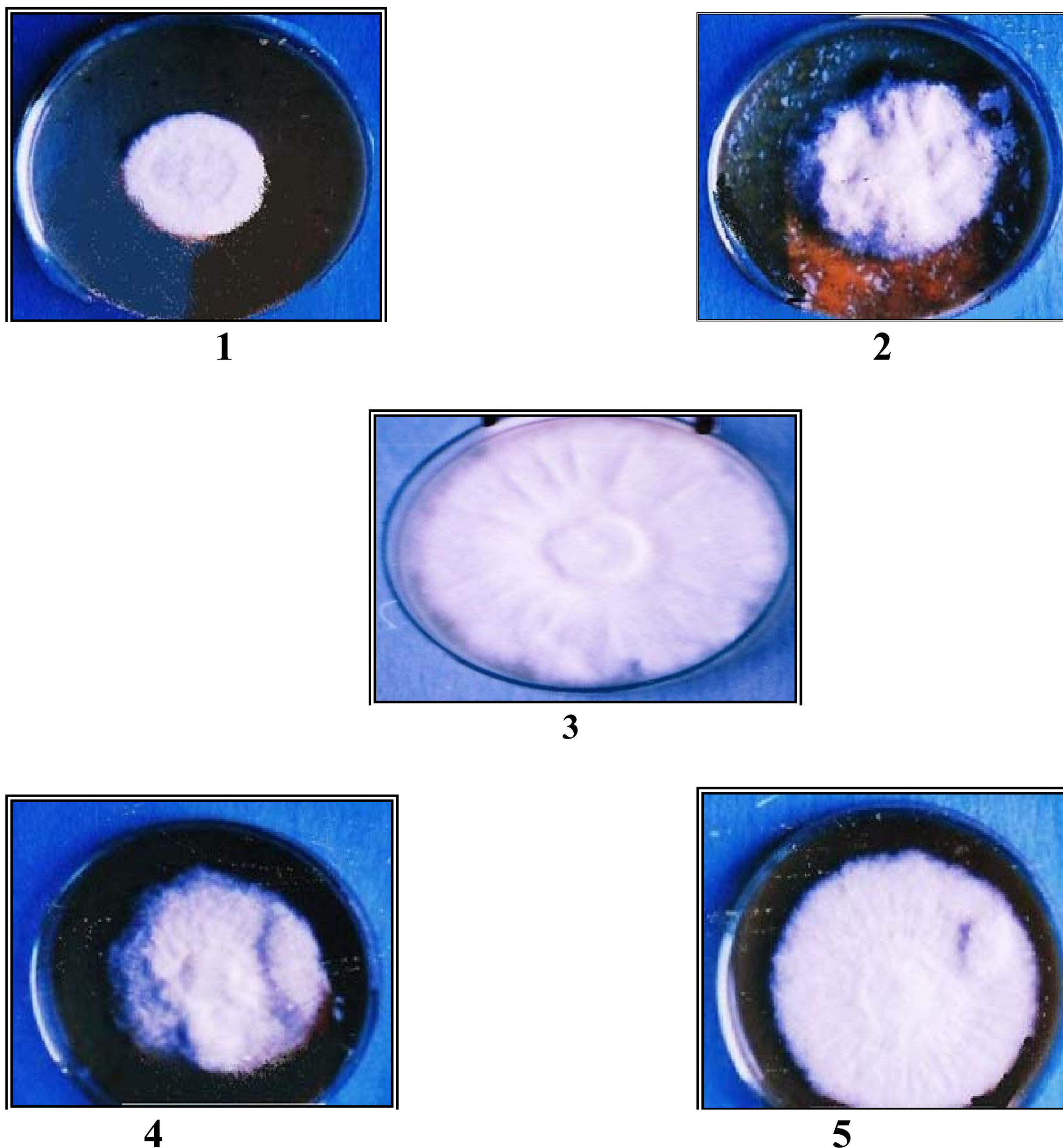


Figure 7: Effect of crude phenolic compounds on the growth of *Trichophyton rubrum*

1=100mg/ml, 2=75mg/ml, 3= control, 4= 50 mg/ml, 5= 25mg/ml.

4.5.6: Effect of crude Alkaloids extract of S. *aegyptiaca* on *Trichophyton rubrum*.

Results show that crude alkaloids extract of *S. aegyptiaca* with different concentrations had significant inhibitory effect (Figure 8 and Table 14). It appears that T. *rubrum* was no significant inhibition effect on these extracts at concentrations (25, 50, and 75) mg/ml, while it has significant inhibitory

effect at 100 mg/ml, with the percentage of inhibition for all concentrations (32.2, 33.3, 42.2, and 77.7) % respectively. A study by Anna *et al*., (2002) suggested that, the crude alkaloid extract of *Mahonia aquifolium* which were screened for inhibitory activity against a variety of dermatophytes and two *Candida* species with concentration ranges (62.5 to 125) mg/ml and dermatophytes were more susceptible more than *Candida*. There is always a possibility that a given extract which is inactive *in vitro* may exhibit properties of pro-drugs which are administered in an inactive form, but their metabolites may be active (Lino and Deogracious, 2006). These results were disagree with those Al-Ani (2005) whom mentioned that low concentration of crude alkaloids of *Fumaria officinals* extract has antifungal activity on *T. rubrum*.

Table 14: effect of crude Alkaloids extract of S. *aegyptiaca* on *Trichophyton rubrum*

Concentration (mg/ml)	Average of colonial diameter (mm)±SE	Percentage of inhibition (%)
0	90.0±0.31a	0
25	61.0±0.5a	32.2
50	60.3±0.76b	33.3
75	52.3±0.76c	42.2
100	20.3±0.76c	77.7

Different letters in the same column significant differs ($p \leq 0.05$) between means.

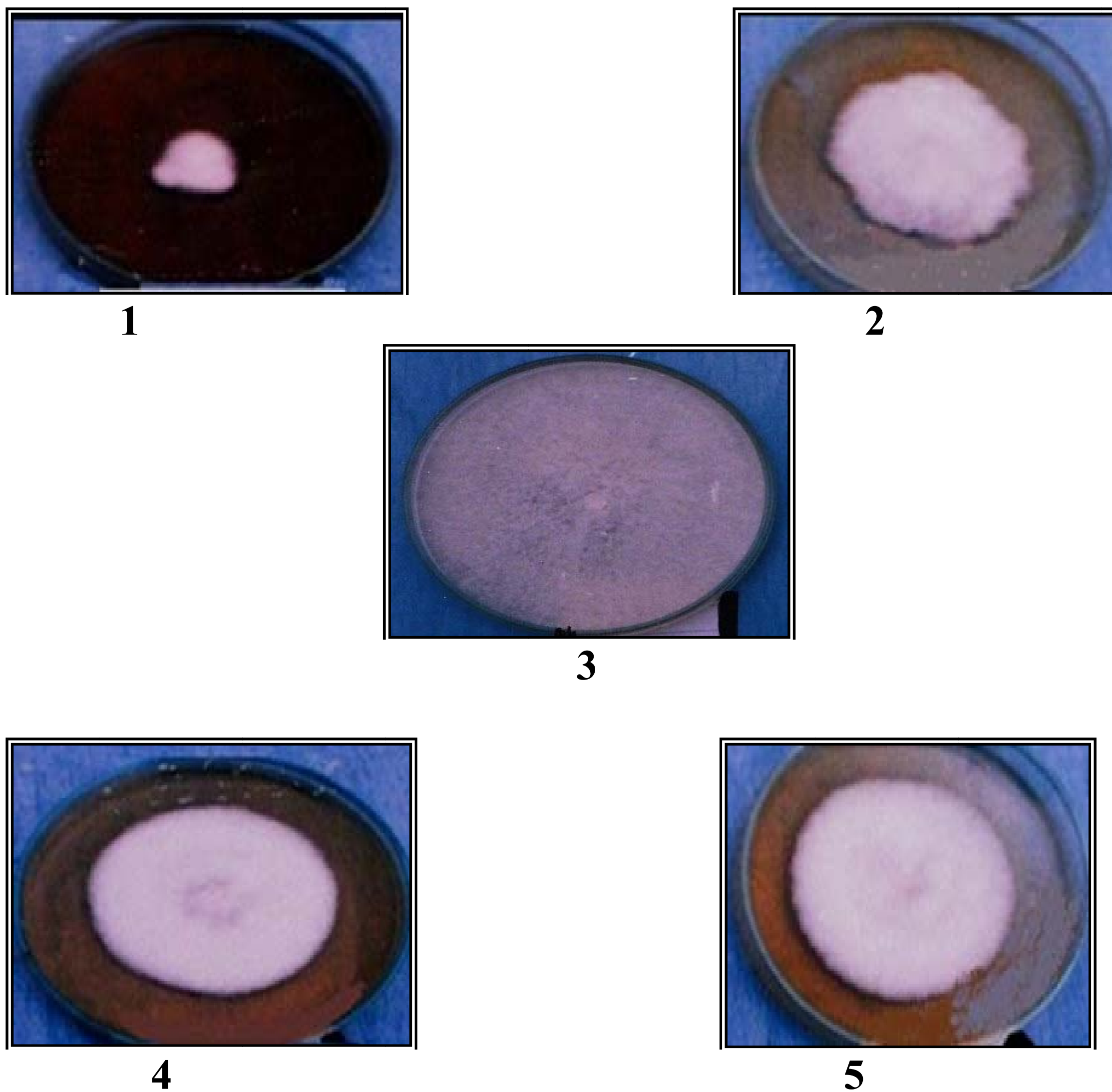


Figure 8: Effect of crude alkaloidal compounds on the growth of *Trichophyton rubrum*

1=100mg/ml, 2=75mg/ml, 3= control, 4= 50 mg/ml, 5= 25mg/ml.

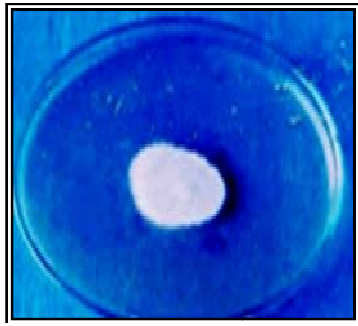
4.5.7: Effect of crude terpenoids extract of S. *aegyptiaca* on *Trichophyton rubrum*

Terpenoids crude extract of *S. aegyptiaca* showed inhibitory effect on growth of *T. rubrum*. Percentage of inhibition of *T. rubrum* at concentration (25, 50, 75 and 100) mg/ml were (8.88, 26.6, 63.3, 65.5 %) respectively (Figure 9 and Table 15). A study by Singh and Singh, (2003) showed that mixture of terpenoids: (beta-sitosterol, alpha-amyrin, lupeol, hexacosanoic acid, ceryl alcohol and hexacosane) were isolated from *Trichodesma amplexicaule* showed antimicrobial activity against selected pathogenic fungi, *T. mentagrophytes* and *P. chrysogenum* .The results of isolated terpenoids were found that hexacosane was more active (inhibition zone=73.39%) against *T. mentagrophytes* and hexacosanoic acid had greater activity against *A. flavus* (Inhibition Zone= 61.56%).

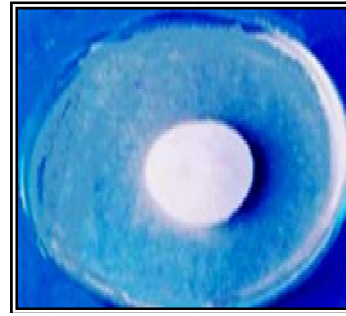
Table 15: The effect of crude terpenoids extract of S. *aegyptiaca* on *Trichophyton rubrum*

Concentration (mg/ml)	Average of colonial diameter (mm)±SE	Percentage of inhibition (%)
0	90.0±0.31a	0
25	82.0±0.76a	8.88
50	66.3±0.76c	26.6
75	32.3±0.57b	63.3
100	31.3±0.76c	65.5

Different letters in the same column significant differs ($p \leq 0.05$) between means.



1



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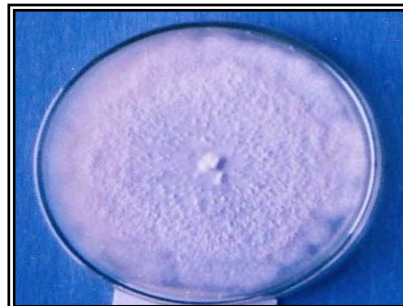

3

4

5

Figure 9: Effect of crude terpenoid compounds on the growth of *Trichophyton rubrum*

1=100mg/ml, 2=75mg/ml, 3= control, 4= 50 mg/ml, 5= 25mg/ml.

4.5.8: Combined effect of Crude (Alkaloids, phenols and terpenoids) extract of S. *aegyptiaca* on *Trichophyton rubrum.*

In this section, the combined effects of the extracted phytochemical (phenol, alkaloid, terpen) were tested against *T. rubrum* at concentration (100 mg/ml). In Figure(10) it is cleared that effect were varied, in the case of the combination of alkaloid and terpen (A+T), alkaloid and phenol(A+P), and phenol and terpen(P+T), the growth of fungus was showed significant inhibition and the percentage of inhibition were (12.5, 41.25, 47.5%) respectively. While, in combination between alkaloid, phenol and terpen (A+P+T), growth showed significant inhibition to *T.rubrum* and percentage of inhibition was (100%) as shown in (Table 16), this suggested the presence of synergistic effects, or additive effects or inhibitory effects of these compounds together.

A study by Shin and Lim (2004) was conducted to the effects of *Pelargonium graveolens* essential oil and its main components citronellol and geraniol combined with antifungal drug ketoconazole against *Trichophyton spp*. the result of their study showed the antifungal effects of ketoconazole against *T.* spp. are enhanced significantly by administering it in combination the essential oil fraction of *P. graveolens* or its main components against *T. soudanense* and *T. schoenleinii*. The exhibition of antifungal activity has been attributed to the presence of plant active compounds (Table 6) which are employed as natural defense mechanism against pathogenic bacteria, fungi, viruses and pests (El-Mahmood and Ameh, 2007).

Manonmani, *et al.,* (1995) were suggested that plant crude extracts was containing a combination of active compounds and these active compounds may acts synergistically, so it could use this advantage for treatment purpose. Kaufman *et al.* (1999) documented how synergistic interactions enhance the effectiveness of phytomedicines. This theme of multiple chemicals acting in

an additive or synergistic manner likely has its origin in the functional role of secondary products in promoting plant survival. For example, in the role of secondary products as defense chemicals, a mixture of chemicals having additive or synergistic effects at multiple target sites would not only ensure effectiveness against a wide range of herbivores or pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses (Wink, 1999).

Table 16: The combined Effect of Crude (Alkaloids, phenols and terpenoids) extractsof *S. aegyptiaca* on *Trichophyton rubrum*

Type of extracts that combined at (100 mg/ml)	Average of colonial diameter (mm)±SE	Percentage of inhibition (%)
0	90.0±0.31a	0
A+T	70.3±0.76a	12.5
A+P	46.0±0.5b	41.25
P+T	40.3±0.76c	47.5
A+P+T	0.0d	100

Different letters in the same column significant differs ($p \leq 0.05$) between means.

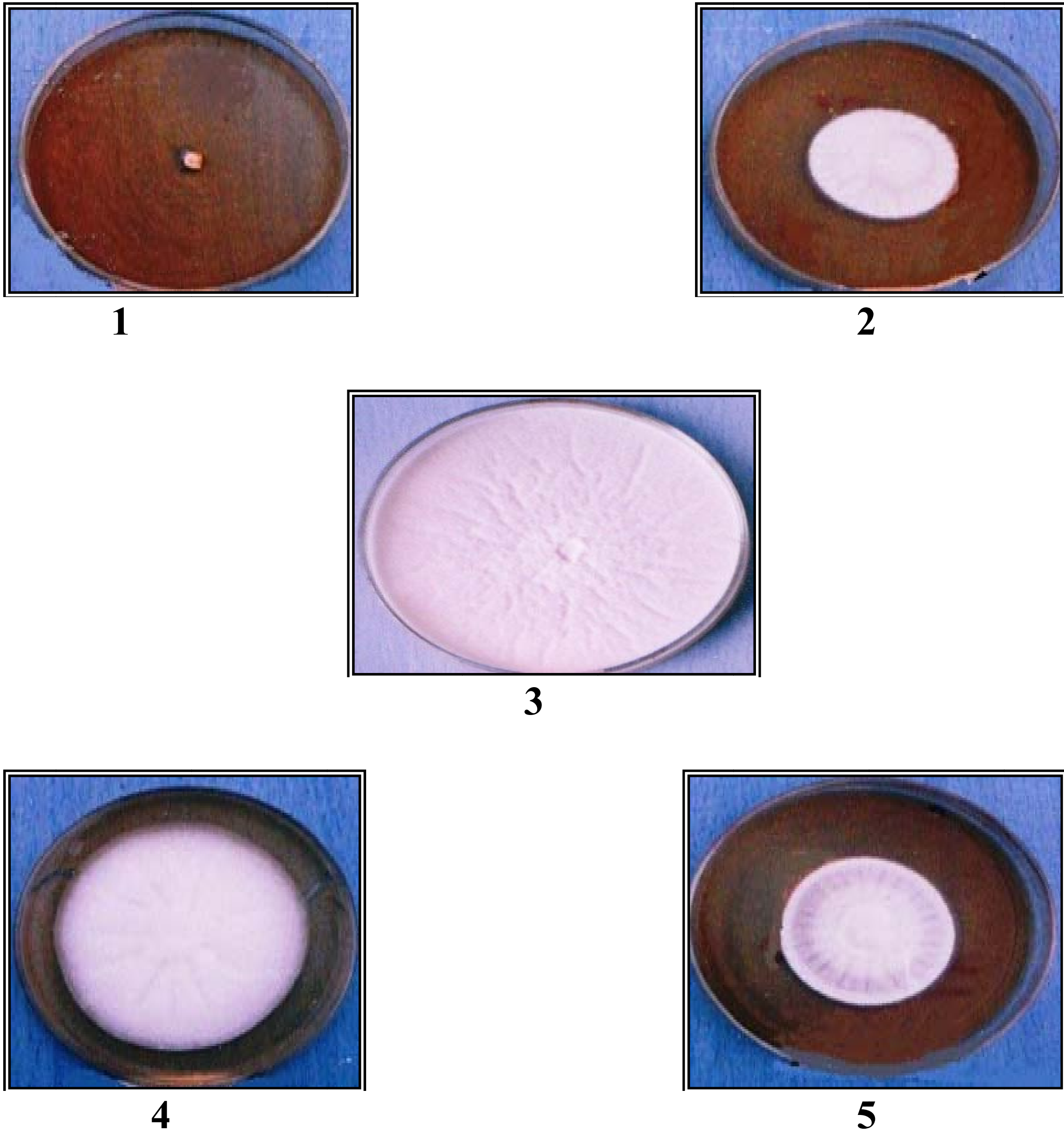

Figure 10: Effect of different combinations of *S. aegyptiaca* crude extracts (phenol, alkaloid, terpen) at (100 mg/ml) on the growth of *Trichophyton rubrum*.

1=A+T+P, 2=P+T, 3= control, 4=A+T, 5=A+P

4.6: Effect of *S. aegyptiaca* crude extracts in the treatment of skin infection caused by *Trichophyton rubrum* in experimental animals.

Certain degenerative changes were pointed after 15 days of infection with *T. rubrum* (Figure 11, 12) due to adherence of the fungal conidia into the keratinophilic layers of the skin producing lesions.The conidia enter the skin through an abrasion, and the hyphae begin to germinate and grow in the stratum corneum. The hyphae invade the hair follicle and enter the cortex of the hair by dissolving the keratin. The conidia and hyphae were carried to the surface by the growing hair that often breaks off. Invasion of the hair causes the shaft to be weak and break resulting in circular, scaly areas with loss of hair (1.7x1.7) as in Figure 11.

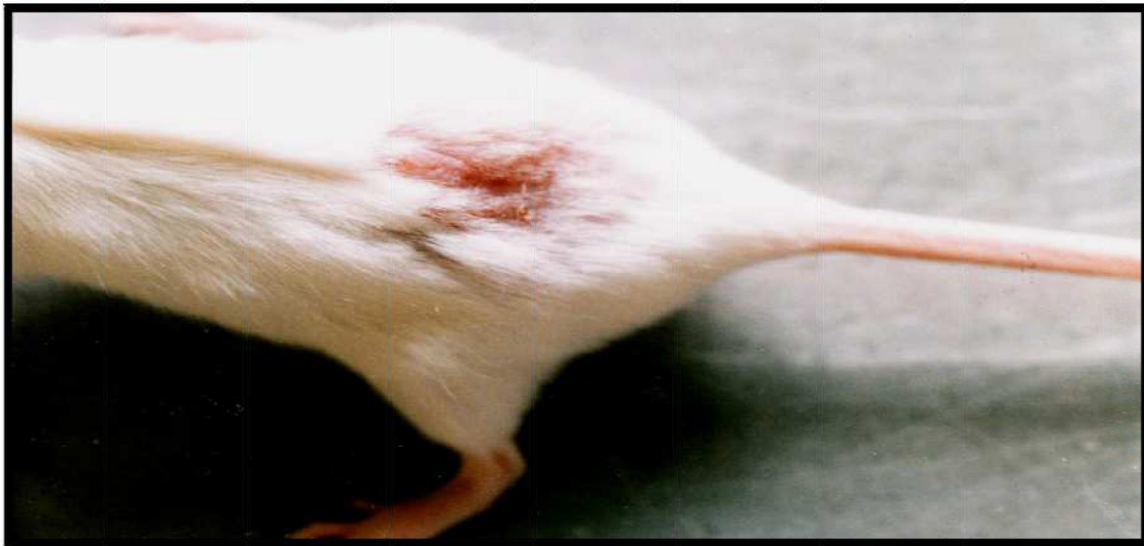

Figure 11: Morphological changes caused by *Trichophyton rubrum*

Proteinases are produced by dermatophytes *in vitro* play an important role in the pathogenesis of fungal infections *in vivo*, it has been suggested by many workers that the pathogenesity of microorganisms is related to production of proteinases which enable them to parasitize tissues such as stratum corneum, nails and hairs (Tsuboi, 1989). The mechanical action of the

invading mycelium penetrating the stratum corneum and the proteinase, keratinases produced by dermatophytes may play an important role in the pathogenesis of fungal diseases *in vivo*. This interaction between the fungal hyphae and its products with the host tissue results in the clinical expression of the disease (Samadani *et al*., 1995).

Skin area infected with *T. ruburm* show irregular margin with boil formation, redness and swelling (2x2) cm (Figure 12). This indicates the invasion of the hyphae into stratum corneum inducing a hyperkeratosis with crust. This represented by elevation of skin due to accumulation of inflammatory cells, this is in turn lead to dilation of the blood vessels causing redness of the skin. Similar changes were observed by Lucky, (1985) on *Trichophyton* infection cause *Tinea capitis*.

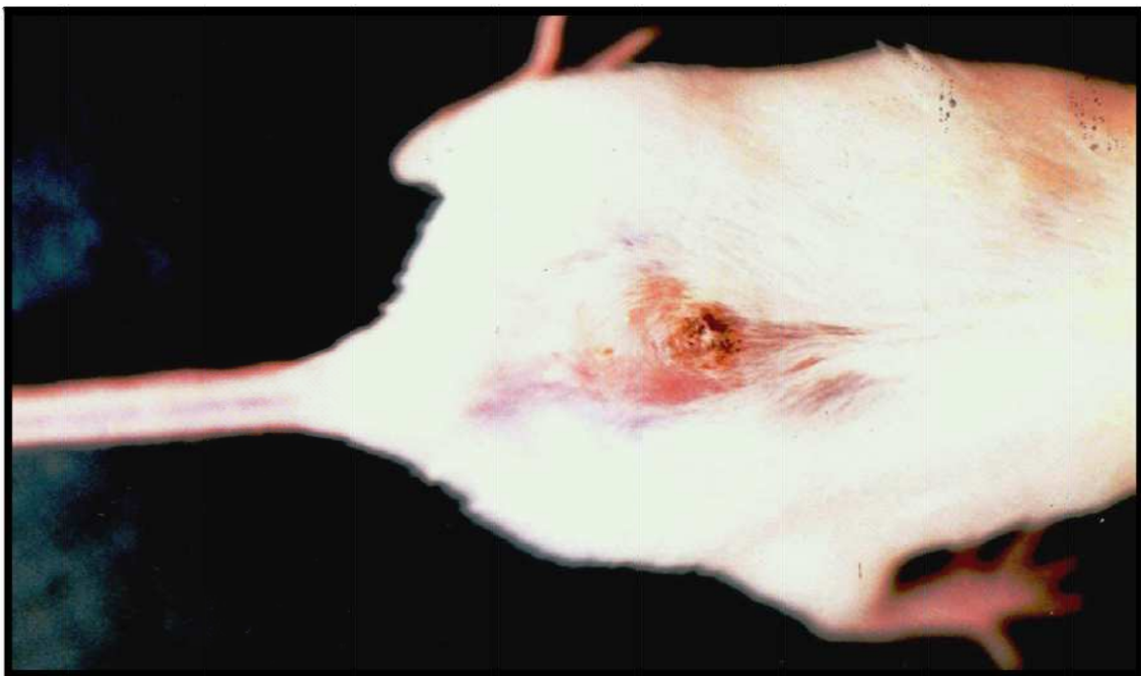

Figure12: Morphological changes caused by *Trichophyton rubrum*

Dermatophytes have the ability to use the keratin as food sources because of keratinase enzyme and infected area have loss of hair, redness area and dilation of blood vessels (Koneman, 1985). The resulting inflammatory

response by the host in the most intense at the area of the recent invasion. Some Dermatophytes produce only mild or no inflammation or immune reaction; in such cases, the organism may persist indefinitely, causing irregular margins of a gradually extending lesion with a scaling slightly raised border (Ohst *et al.,* 2004). Lesions usually appears as erythematous area with fine scales, often in circular patterns,the scales represent increased epidermal turnover in response to the inflammation(Weitzman and Summerbell, 1995). After the treatment with *S. aegyptiaca* extracts regenerative change were observed in all treated mice. The crusts tend to drop-off spontaneously after 9 days of the treatment leaving a white scar, usually slightly depressed along its entire length. This initial depression of the healing scratch line disappeared and incision was covered with newly formed epithelium and the hair as seen in (figure 13, 15) which represented the skin infected and treated with the combination of the (alkaloid, phenol and terpens) extracts at (100 mg/ml), the regeneration was gained after the treatment with extract. The dermatophytes have ability to invade keratinized tissue (skin, hair and nails) but are usually restricted to nonliving cornified layer of the epidermis because of their inability to penetrate viable tissue of an immuncompetent host (Serrano, 2004). Chronic dermatophtosis is mostly caused by *Trichophyton ruburm,* and there is some evidence that infection produced by the fungus suppresses or diminishes the inflammatory response (Santos, 2005). Most fungi can survive a wide range of temperature (from 10° C to 40° C), the human body temperature of 37° C is quite high for the survival and growth of most fungi with exception of *Candida albicans*, which usually grows well in a laboratory temperature of 37C°, and the dermatophytes (ringworm), which grow well at 28.3 C° to 30 C°, So, the human body is capable of being their host (Elewski, 2000).

In Figures (13, 15) it's observed that the skin of mouse treated with *S. aegyptiaca* showing slight remaining of previous infected area, growth of hair and still showing mark between regenerative area and normal area. Physical examination involves observing the skin, nails, hair and mucous membranes. Noting color, size of lesions, moistness, dryness, texture and anatomic location (Gupta and Shear, 1999).

In Comparison these results with the animals that infected with *T. rubrum* that treated with mycodin ointment, after the 12th day of the treatment, slower regenerative changes were seen. The crust tends to drop-off spontaneously and incision which was covered with area less than those that covered the area treated with *S. aegyptiaca* extracts.

In group treated with mycodin, redness still persist with crusting and slightly loss of hair (Figure 14) indicated that *S. aegyptiaca* extracts is more effective than mycodin in healing the infected area suspecting that time for healing by *Suaeda* extracts is less than that in mycodin ointment which are also another fact for the activity of this plant extracts.

There are four defense mechanisms against dermatophytes infection includes:

- 1-The skin's natural barrier function.
- 2-Skin turnover.
- 3-Serum inhibitory factor.
- 4-Cellular immune system (type IV delayed hypersensitivity reaction) (Czaika, *et al.,* 1998).

Pharmaceutical studies of antifungal agents on drugs are classified into:

- Drug that disrupt the cell membrane.
- Drug that inhibit the mitosis.
- Drug that inhibit deoxyribonucleic acid (DNA) synthesis.

(Laurence *et al*., 1999).

Three general modes of action of different plant extract were recognized as follows:

- Inhibit of microbial cell wall formation or biosynthesis of some essential protein.
- Disruption of deoxyribonucleic acid (DNA) metabolism.
- Alteration of normal function of cellular membrane.

(Tyler *et al*., 1999).

Some drugs can be administered alone or as poly-pharmaceutical (following the principle of therapeutics) in the form of decoction, tablets, infusions, powders, confection, electuaries, and syrups. In the field of preventive and primary health care, it is notable that most of Unani medicines are not liable to produce harmful side effect in contrast with some chemicals or synthetic drugs (Chopra *et al*., 1978).

Figure 13: External features of mice infected with *Trichophyton rubrum* and treated with *S. aegyptiaca* crude extracts.

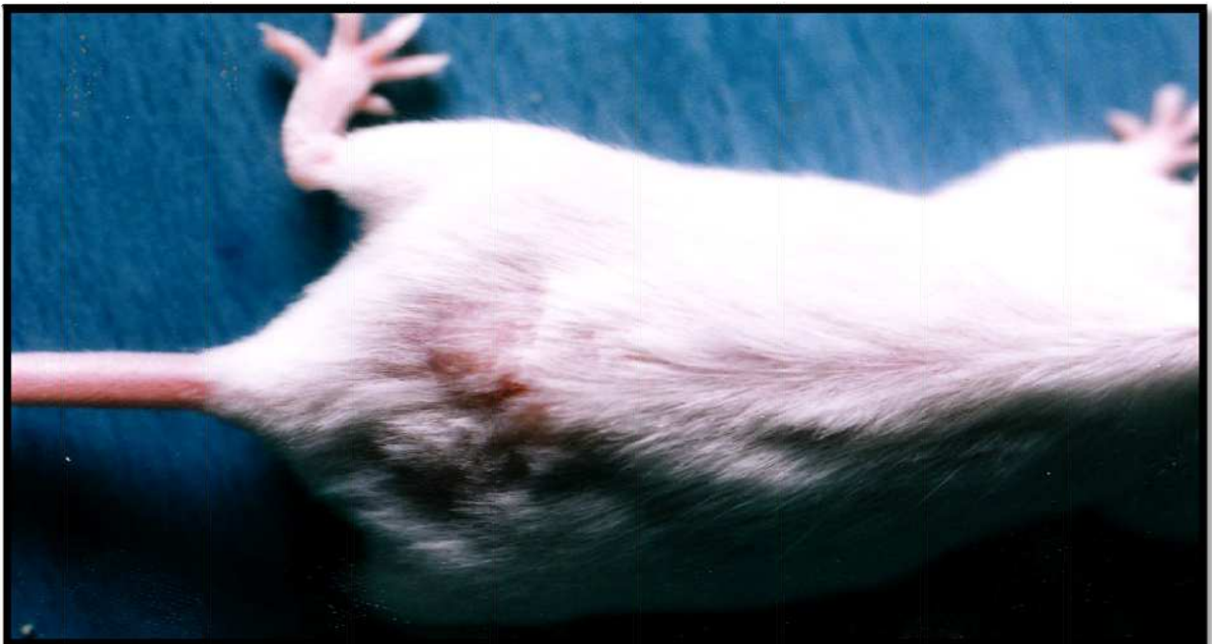

Figure 14: Morphological repair of the animal infected with *T. rubrum* and treated with mycodin ointment and show loss of hair

Figure 15: Morphological repair of the animal infected with *Trichophtyon rubrum* and treated with *S. aegyptiaca* crude extracts.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

- *S. aegyptiaca* contain different active compounds including: phenols, alkaloids, flavonoids, terpens, resins, glycosides, saponins and tannins.

- Agar well diffusion test showed wider inhibition zones than papers disc diffusion test.

- Combination between three crude *S. aegyptiaca* extracts (alkaloid, phenol, terpen) showed inhibitory against pathogenic microorganisms.
- The plant crude extract have antimicrobial effects on pathogenic microorganisms.
- *S. aegyptiaca* extracts have the ability to heal skin that infected with *T. rubrum* and it's more efficient than mycodin ointment.

Recommendations

- Qualitative and quantitative study on separation of different active compounds present in *S. aegyptiaca*.
- Study the activity of *S. aegyptiaca* as antimicrobial agent against other microorganism.
- Further studies are needed on the effect of plant extract on the animal and human chromosome.
- Further research on the pharmacological activity of this plant including cytotoxicity and antitumor activity.
- Using some parameter for detection the effect of plant extract on the immune system.

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الخلاصة

- أجريت سلسلة من التجارب لغرض تقييم استعمال مستخلصات الأجزاء الهوائية الخام لنبات الطرطيع (*Suaeda aegyptiaca*) ضد الأحياء المجهرية الممرضة خارج الجسم الحي، فضلاعن دراسة الفعالية العلاجية لخليط مستخلصات (القلويدات، الفينولات والتربينات) الخام داخل الجسم الحي على جلد الفئران المصابة بفطر *Trichophyton rubrum* .

- بينت الدراسة الكيميائية لنبات الطرطيع احتوائه على عدد من المركبات الفعالة والتي كانت:الفينولات، القلوانيات والتربينات.
- أظهرت الدراسة الأولية للفعالية الحيوية للمستخلصات (المائية والايثانولية) لنبات الطرطيع أن المستخلص الايثانولي يمتلك فعالية تثبيطية لبكتريا *Stapylococus aureus, Staphylococcus epidermidis* *Klebseilla pneumoniae*، في حين كان للمستخلص المائي تأثيرا على *Staphylococcus aureus,* فقط ،فيما كانت *Candida albicans*، *Escherichia .coli* و *Trichophuton rubrum* مقاومة لكلا المستخلصين.

- استخلصت الفينولات، التربينات والقلويدات من الأجزاء الهوائية لنبات الطرطيع،و حضرت تراكيز مختلفة منها (25، 50، 75 ، 100) ملغم/ مللتر.
- لدى دراسة تأثير المستخلصات المخلوطة مع بعضها البعض باستخدام تركيز(100) ملغم/مللترلكل منها. أظهرت النتائج تفاوت في فعاليتها مقارنة مع فعالية هذه المركبات لوحدها. مما يوصي بإمكانية وجود أما فعل تآزري أو فعل مضاف أو فعل مثبط.
- أدت الإصابة *بالفطر* *Trichophyton rubrum* ألى حدوث تغيرات مظهرية في جلد الحيوان شملت ظهور منطقة محرشفة وحافات غير منتظمة مع تكون خراجات ، احمرار وانتفاخ وسقوط الشعر.

- أظهرت نتائج المعالجة بخليط المركبات الفعالة (الفينولات، التربينات والقلويدات) الخام بتركيز (100) ملغم/مللتر إلى حدوث تغيرات بالجلد تمثلت بنقصان التقشر تدريجيا تاركة ندب بيضاء.

- اتضح بعد تسع أيام من العلاج بمستخلصات الطرطيع الخام أن المنطقة غطيت بنسيج ظهاري جديد مع ظهور الشعر، وعند مقارنتها بالنتائج التي اعطاها مرهم Mycodin المضاد للفطريات فقد كانت التغييرات البنائية للمستخلصات أبطا حدوثا وذلك من خلال انخفاض نسبة التقشر ونمو الشعر خلال نفس الفترة الزمنية مما يؤكد أفضلية مستخلصات الطرطيع بصفتها المضادة للفطريات.

الإهداء

غاية ألحمد والشكر لولي التوفيق والفضل العظيم   الله(عز وجل)

إلى كل ذرات ألتراب ألغالية ألمخضبة بدماء الأبرياء   وطني العراق

إلى الروح الخالدة.. إلى من غرسني ولم يشهد أوان عطائي   والدي (رحمه الله)

إلى ملكة حياتي من علمتني المطاولة والصبر والإصرار على التفوق   والدتي الحبيبة

إلى من زرع ألطموح ورغبتي أن أكون مثلهم   أساتذتي

إلى ربيع عمري..إلى نور حياتي.. إلى الشموع التي تنير لي دربي   أخواني وأخواتي

و إلى كل من أصبحت أشغل تفكيره وهمه أن انهي هذا ألبحث ....

اهدي ثمرة جهدي المتواضع

رندة

بِسْمِ اللهِ ألَّرحمَنِ ألرَّحِيم

وَأنزَلَ الله عَلَيْكَ ألْكِتَابَ وَألْحِكْمةَ وَعَلَّمَكَ مَالمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللهِ عَلَيْكَ عَظِيماً

صَدَقَ **الله** ألعَظِيم

سورة النساء

أية 113

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

تأثير مستخلصات نبات الطرطيع خارج وداخل الجسم الحي على بعض ألاحياء المجهرية

رسالة
مقدمة إلى كلية العلوم/ جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الإحيائية

من قبل

رندة محمد ضاحي المجمعي

بكالوريوس تقانة إحيائية / كلية العلوم
جامعة النهرين(٢٠٠٥)

شوال 1429

تشرين الأول ٢٠٠٨