Republic of Iraq
Ministry of Higher Education and
Scientific Research
Al-Nahrain University
College of Science
Department of Biotechnology

Activity Of Some Secondary Metabolites From *Tagetes patula* L. Flowers Against Microbial Infection

A thesis

Submitted to the College of Science, Al-Nahrain University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

By

Farah Dawood Salim Al-Saadi

B.Sc., Biotechnology, College of Science, 2006

Al-Nahrain University

Supervised by

Dr. Kadhim M. Ibrahim
Professor

Dr. Munira Ch. Ismail
Professor

November
2009

Zo-Alkada
1430

Supervisor(s) certification

We, certify that this Thesis " Activity of Some Secondary Metabolites from Tagetes patula L. Flowers against Microbial Infection "
Was prepared by "Farah Dawood Salim " under our supervision at the Department of Biotechnology, College of Science, Al-Nahrain University, as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:
Name: Dr. Kadhim M. Ibrahim
Scientific degree: Professor
Date:

Signature:
Name: Dr. Munira Ch. Ismail
Scientific degree: Professor
Date:

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature
Name: Dr. Kadhim M. Ibrahim
Scientific degree: Professor
Title: Head of Biotechnology Department
Date:

Committee Certification

We, the examining committee certify that we have read this thesis entitled " Activity of Some Secondary Metabolites from Tagetes patula L. Flowers against Microbial Infection " and examined the student "Farah Dawood Salim " in its contents and that in to our opinion, it is accepted as a thesis for the degree of Master of Science in Biotechnology.

(Chairman)
Signature:
Name: Dr. Khulood W. Al. Samarraei
Scientific degree: Professor
Date:

(Member)
Signature:
Name: Nabeel K. Al-Ani
Scientific degree: Assist. Prof.
Date:

(Member)
Signature:
Name: Dr. Swosan H. Ithmian
Scientific degree: Assist. Prof.
Date:

(Member)
Signature:
Name: Dr. Kadhim M. Ibrahim Ismail
Scientific degree: Professor
Date:

(Member)
Signature:
Name: Dr. Munira Ch.
Scientific degree: Professor
Date:

I hereby certify upon the decision of the examining committee.
Signature:
Name: Dr. Laith Abdul Aziz Al-Ani
Scientific degree: Assistant Professor
Address: Dean of the College of Science
Date:

Acknowledgements

First praise goes to almighty "Allah" for giving me the time, strength, faith and helped me to complete my thesis.

Sincere appreciations and grateful to my supervisors Professor Dr. Kadhim M. Ibrahim and Professor Dr. Munira CH. Ismail for suggesting the topic of my thesis, their tremendous help, efforts and support giving me a chance to add a letter in the science encyclopedia.

It is a pleasure to thank all staff members and employers of Biotechnology Department at Al-Nahrain University for their friendship and support.

Finally, I so thankful to my father and mother for being the shoulder and shelter, my brother (Mohammad), and my sisters (Halah and Rasha). May Allah bless you all.

Farah

Summary

In an attempt to treat infection of mice skin, and investigate the antimicrobial effect of bacterial system (G-system) using essential oil and methanol extract of *Tagetes patula* flowers (Qadifah), several experiments were carried out. These extracts were investigated for the presence of some active secondary metabolites and their antimicrobial activity. Tagetes methanol flowers extract contained phenols, flavonoide, alkaloids, saponins, and terpenes. This extract showed an inhibitory effect better than essential oil (undiluted) against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Klebseilla pneumoniae*, and *Candida albicans*. *Escherichia coli* was resistant to both essential oil and methanol extracts. Essential oil (undiluted) and methanol extract (50mg/ml) showed better inhibitory activity against *S. aureus*, thus those extracts were applied on laboratory mice skin. Essential oil and methanol extract had antibacterial, anti-inflammatory and healing effect as compared with the commercial skin ointment (Samacycline) on mice skin infected with *S. aureus*.

Methanol extract was rich in flavonoides, and showed no cytotoxic effect on the G-systems [*Bacillus spp.* (G3), *Arethrobacter spp.* (G12), and *Brevibacterium spp.* (G27)]. The interaction treatments (per, simu, and post) of the drug (Methotrexate) at the concentration 50µg/ml and methanol extract at concentration 50mg/ml were employed to reduce the activity of drug on the bacterial system.

List of contents

List of tables

List of figures

List of appendices

List of abbreviations

Abbreviation	Full name
D.W.	Distilled water
DHFR	Dihydrofolate reductase
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
G3	*Bacillus spp*.
G12	*Arthrobacter spp*.
G27	*Brevibactrium spp.*
hrs	hours
L	Liter
LSD	Least Significant Differences
MTX	Methotrexate
PBS	Phosphate Buffer Saline
rpm	rotation per minute
ROS	Reactive Oxygen Species
v/v	volume to volume
w/v	weight to volume

Chapter one

Introduction and Literature Review

1.1- Introduction

Herbs and spices have a long history for both culinary use and health benefits as well as acting as preservatives. Millions of people in the third world use herbal medicine. People in Europe, the United Kingdom and the United States of America are turning to alternative medicines to some extent because of the side-effects induced by powerful, synthetic, allopathic drugs **(**Hemphill and Cobiac, 2006).

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 store of antimicrobial drugs prescribed by physicians (Hemphill and Cobiac, 2006).

Research on the medicinal plants has 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 antimicrobial, antioxidant, and antitumoural agents (Azhar, 1998).

Edward and Gilman (1999), and Melissa (2007) stated that tagetes species, popularly known as marigold 'Mary's gold' because they were considered to be the Virgin Mary's flowers. Marigolds are categorized into two basic types: the large-flowered African marigold *(Tagetes erecta*) and the smaller-flowered French marigold (*Tagetes patula*).

Saleem *et al.,* (2004), mentional *Tagetes patula* (Asteraceae), locally known as Qadifah or Jafri grows in gardens as ornamental plant across the globe and used for the preparation of high grade perfumes in France.

The majority of *Tagetes patula* is related to its content of important active constituent's mainly essential oil which is known for its antibacterial and insecticidal properties. Carotenoids are used as food additives, possessing anticancer and antiageing effects; thiophenes with a marked biocidal activity. Flavonoids have pharmacological properties (Szarka *et al*., 2007).

Digrki *et al.,* (1999), reported that *Tagetes patula* flower extracts have a wide range of biological activities, such as: antispasmodic, emmenagoguic, fungicidal, sudorific, and vermifugal.

The plant extract is used for treatment of athlete's foot, ringworm, and absence of menstruation (Ballesta *et al.,* 2008).

Skin infections are often the result of a break in the integrity of the skin. Bacterial skin infections technically have specific names based on their origin and extent, but even in the medical community, the common term is boil or abscess (Daniel *et al*., 2002).

According to Jolivet *et al*., (1995), and Iqbal, (2003), Methotrexate (MTX) was used since 1951 as chemotherapeutic agent. It is a foliate antagonist which kills the proliferating cells by inhibition the activity of the dihydrofolate reductase enzyme (DHFR).

The main problems in chemotherapy treatment is the side effect which may lead to toxicity or even death, and therefore several studies have been made to overcome this problem such as making interactions between herbs and drugs.

Due to the importance of this locally grown plant as a potential source for phytochemicals, this research work was aiming to:

1. Detect some active compounds in flower extracts of the *Tagetes patula*.
2. Study the antibacterial and antifungal activity of extracts (*in vitro*).

3. Administration of essential oil and methanol extract of *Tagetes patula* on infected mice skin in an attempt to treat the infection (*in vivo*).

4. Determine the antimicrobial effect of *Tagetes patula* against the toxic effect of methotrexate (*in vitro*).

1.2- Literature Review

1.2.1- Herbal medicine

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Herbal medicine is also known as botanical medicine, medical herbalism, herbology, and phytotherapy. Herbs and spices used by humans yield useful medicinal compounds (Tapsell, 2006; Ang-Lee *et al.,* 2008).

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 of a great significance in medicine. A number of studies have been conducted in different countries to prove such efficiency (Shapoval *et al*., 1996).

World Health Organization (WHO) noted that the world's population depends on traditional medicine for primary healthcare. Many plants were used because of their antibacterial, antifungal, and antioxidants character, due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances; for example, phenolics, alkaloids and terpenoids (Scalbert, 1991; Altwegg *et al.,* 1999; Prabuseenivasan *et al*., 2006).

Many conventional drugs originated from plant sources, examples include aspirin from willow bark, digoxin from foxglove, and morphine from the opium poppy **(**Reverned, 1998).

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).

The use of a mixture of natural products to treat diseases 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).

The other advantage of the treatment with plant drugs is the reduction of the side effects that often occur with the synthesized medicine. This may be due to the lower concentration of the active compounds found in the plants that the human body would need (Tsuda *et al.,* 2004).

1.2.2- The studied plant

1.2.2.1-Tagetes

Tagetes is a genus of about 60 species of annual and perennial herbaceous plants in the daisy family (Asteraceae). They are native to the area stretching from the Southwestern United States into Mexico and south throughout South America (Gupta *et al.,* 2007).

According to Edward and Gilman, (1999) Tagetes includes several species of economic importance, namely: *Tagetes erecta*, *Tagetes filifolia*, *Tagetes lacera*, *Tagetes lucida*, *Tagetes minuta*, *Tagetes patula*, and *Tagetes tenuifolia*.

Several species possess antibacterial and antihypertensive properties. They mentioned in folk medicine for relief of toothache and chronic rheumatic, while others have been utilized to desalinize irrigated farmland. Investigation of

certain species of this family revealed that they contain triterpenoids, saponins, phenolics, and alkaloids (Szarka *et al*., 2007).

1.2.2.2- *Tagetes patula* L. (Qadifah)

The plant common names are: French marigold [England], tudentenblume [German], Amapola amarilla [Spanish], Clavel De Los Muertos, Copetes [Spanish], Copetillo [Spanish], Copada, Flewurs Souci, Dwarf, Garden Marigold, Jafari, Qadifah, and Souci (USDA, 2008).

T. patula is an annual, a small bushy plant about (15-30) cm in height, flowers may be yellow, orange and a unique bronze color. It flowers from December to July, and the seeds ripen in November. The scented flowers are hermaphrodite (have both male and female organs) and are pollinated by insects (Figure 1), leaves are dark green, deeply divided and have unpleasant aromatic fragrance (Scheper, 2003).

The French marigold is native to Mexico, and Nicaragua. In addition, it has been cultivated in Europe, Iraq, India, China, Argentina, and Colombia, and in the United States for its medicinal properties and as a flowering ornamental plant (USDA, 2008).

1.2.3-Taxonomy of *Tagetes patula* (Gupta *et al.*, 2007)

- Kingdom: Plantae
- Division : Magnoliophyta
- Class : Magnoliopsida
- Order : Asterales
- Family : Asteracea

- Tribe : Tageteae
- Genus : Tagetes
- Species : patula L.

Figure1: Tagetes (*Tagetes patula* L.) photographed by the researcher.

1.2.4- History and popular uses

Tagetes species have been used by humans for hundreds of years. Ancient Egyptians valued it as a rejuvenating herb, Persians and Greeks garnished and flavoured food with the flower petals. Hindus used it to decorate temple altars. During the superstitious Middle Ages, it was used in potions that gave young maidens the ability to discover who they would marry. In Caribbean, Chinese, and Peruvian folk medicine, Tagetes was used for the treatment of angina, high blood pressure, cough, diarrhea, diuresis, fever, inflammation, rheumatism, and food poisoning (Dimo *et al.,* 2003).

During the Civil War, American doctors relied on its antiseptic properties and packed open wounds with marigold leaves. Medieval monks prescribed it for bowel problems, liver complaints, insect and snake bites (Hadjakhoondi *et al.*, 2005).

Europeans have long been used it to flavor soups and stews and to colour butter and cheese (Mills, 2000).

During the 12^{th}, 13^{th}, and 14^{th} centuries, it was used to improve eyesight, clearing the head, and encouraging cheerfulness. It was employed in 17^{th} century to strengthen the heart, and treatment of small pox and measle diseases (Hansel and Stichergger, 1999).

Tagetes is thrown over graves and altars (Chevallier, 1996). It is also used in feeding chickens, giving a bright yellow colour to the yolks and to the skin of the chickens themselves (Hadjakhoondi *et al.*, 2005).

1.2.5- Secondary metabolites

Secondary metabolites are variant and complex chemical compounds widely investigated by organic chemists because of their interesting chemical diversity (Abuharfeil *et al*., 2000). Scientists classified them according to their chemical structure. Others classified them 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).

Different secondary metabolites are detected in *T. patula* including essential oil, terpenoids, flavonoids, alkaloids, polyacetylenes and fatty acids. In addition to the above mentioned compounds, the genus Tagetes is rich in xanthophylls, as

well as smaller levels of thienyls, pyrethrins, and ketones (Assunta, 2006; Francisco, 2008).

T. patula flowers are dominated by mono- and sesquiterpenes [e.g sesquiterpene hydrocarbon β-caryophyllene 50.2 %] (Szarka *et al*., 2007).

The petals are containing (9-22) % flavonoids (Vasudevan *et al.,* 1997). Flavonoids constitute large group of natural compounds, including patulitrin, patuletin, and quercetagetin 7-*O*-glucoside (Faizi *et al.,* 2008; Pauline *et al.,* 2008).

Kasumov (1991) and Richard (2004) isolated carotenoids from air dried *T. patula* flowers. These comprise galenine, lutein, zeaxanthin, lycopene, *β*-carotene, *α*-carotene, and *y*-carotene. Further, carotenoids can be distinguished into those with provitamin A activity, and those without provitamin A activity. Piccaglia *et al.,* 1998 stated that lutein and zeaxanthin do not have any provitamin A activity, but *β*-carotene has provitamin A activity.

The essential oil extracted from aerial parts of *T. patula* yielded 0.17%. The main compounds of the oil were piperitone, *trans*-β-ocymene, erpinolene, β-caryophyllene, myrcene, limonene, cis-β-Ocymene, linalool, β-Farnesene, epoxy-ocymene, germacrene-D, and bicyclogermacrene (Dharmagadda *et al.,* 2005; Sagar *et al.,* 2005).

Extracts of roots and hairy root cultures were found to contain sulfur-containing thiophene structures (Szarka *et al*., 2007).

Recent studies describe the detection of new compounds along with well known citric acid, its trimethyl and dimethyl derivatives, pyridine hydrochloride, 2,2',5',2''-terthiophene, dimethyl mallate, malic acid and 2-formyl, 5-ydroxymethyl furan from roots of *T. patula* (Mohanasun *et al.,* 2007).

1.2.6- Tagetes biological activity

T. patula shows a wide range of antimicrobial activities (Johnson *et al.,* 2000; Saleem *et al.,* 2004; Faizi *et al.,* 2008). The tagetes oil showed a very strong activity against important human pathogenic Gram-positive bacteria and Gram-negative bacteria (Vasudevan *et al.,* 1997; Singh *et al.,* 2005). Significant activity was noticed against three phytopathogenic fungi: *Botrytis cinerea, Fusarium moniliforme* and *Pythium ultimum,* and also against yeast infection such as *Candida albicans* (Aroo *et al.,* 1995; Djehuty, 2007).

T. patula essential oil has larvicidal activity against three mosquito species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefaciatus* (Dharmagadda *et al*., 2005).

Flower petals of *T. patula* have been reported to possess anti-inflammatory activity (Kasahara *et al*., 2002).

Pharmacological evaluation of chemical constituents isolated from *T. patula* showed that its flavonoids and glycosides possess cholagogic activity, patuletin and patulitrin as on active antibacterial against *Corynebacterium spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, and *Micrococcus luteus*, and also antispasmodic; steroids and triterpenes are antimicrobial in nature. Polyacetylenes have cercaricidal activity, while thiophene, its bi and trimers are responsible for antibacterial, antifungal, nematocidal and insecticidal activities, also potential alternatives to synthetic pesticides (Ivancheva and Zdravkova, 1993; Vasudevan *et al*., 1997; Margl *et al.,* 2002; Saleem *et al.,* 2004; Faizi *et al.,* 2008; Francisco, 2008).

Secretions from roots of growing plant are insecticidal. It is used for amending soil infected with *Meloidogyne incognita* on tomato roots (Ploeg and Antoon, 2000). These secretions affect nematodes, and to some extent against slugs (Vasudevan *et al.,* 1997; Marris, 2000).

T. patula contains lutein, and zeaxanthin which are effective antioxidants, protecting important bimolecular and cells against damage caused by free radicals. Diets high in fruit and vegetables provide lutein and zeaxanthin. These two carotenoids contribute to the health benefits observed with such diets, for example in relation to heart disease and cancer (Heinrich, 2003). Lutein may protect against certain eye diseases (Krinsky, 2003).

Acute toxicity studies of tagetes extract in rats and mice showed that it is non toxic but causes minimal skin irritation (Rose *et al.,* 2006), and no sensitization or phototoxicity, not mutagenic and not genotoxic on *Drosophila melanogaster* (Mohanasun *et al.,* 2007).

1.2.7- Tagetes medicinal uses

In current herbal medicine, *T. patula* is aromatic, digestive, diuretic, and sedative (Marris, 2008). It is used to treat indigestion, gastritis, intestinal worms, colic and as an emmenagoguic (Rose *et al*., 2006). It helps with chest infection, coughs and catarrh, dilating the bronchi, facilitating the flow mucus and dislodging congestion (Bond and Goldblat, 2007).

It is a good remedy for the pain and swelling caused by wasp and bee stings. A lotion made from tagetes flowers is useful for wounds, easing muscular pain and painful joints (Grieve, 2007). In addition to the therapeutic efficacy of tagetes extract ointment on the epithelialization of lower leg venous ulcers (Duran *et al.,* 2005), and used to sore eyes and rheumatism (Kirtikar and Basu, 2000).

The plant has been reported to possess hepatoprotective, antiseptic, and blood purifying activity (Vasilenko *et al*., 1990; Saleem *et al.,* 2004).

It was suggested that essential oil extracted from tagetes plant is useful in cases of skin infections, fever, vomiting, and has healing effect on wounds, cuts,

calluses and bunions. It helps to inhibit fungal infections on the skin such as athlete's food (Khan *et al.,* 1996).

Analysis of *T. patula* has discovered the presence of constituents that may affect the blood pressure (Saleem *et al.,* 2004). Citric acid, dimethyl citrate and malic acid have been detected as hypertensive principles while pyridine hydrochloride as hypertensive constituent of plant roots (Kagan, 1991; Schneider *et al.,* 1998).

Tagete is considered today to be relevant in the preparation of cardiovascular drugs (Voutilainen *et al.*, 2006).

1.2.8- Skin infections

1.2.8.1- Bacterial skin infections

There are many types of bacteria; most of them cannot cause disease. Many species even play beneficial roles producing antibiotics and foodstuffs. Soil living bacteria perform many essential functions in the biosphere, like nitrogen fixation. Our bodies are covered with commensally bacteria that make up the normal flora. Bacteria such as some *Staphylococcus spp.*, *Corynebacterium spp.*, *Brevibacterium spp.*, and *Acinetobacter* live on normal skin and cause no harm. Propionibacteria live in the hair follicles of adult skin and contribute to acne (Daniel *et al*., 2002).

The classification of bacterial skin infections (pyodermas) is an attempt to integrate various clinical entities in an organized manner. It is caused by the presence and growth of microorganisms that damage host tissue. The extent of infection is generally determined by how many organisms are present and the toxins they release (Roberts and Chambers, 2005).

Common bacterial skin infections include boils, cellulites, erysipelas, impetigo, folliculitis, furuncles, carbuncles, and hot tube folliculitis. Boils which is skin infection caused by *S. aureus* is quite common. Cellulite is an infection of the deeper layers of the skin and the connective tissues below the skin's surface that has poorly demarcated borders. People with cellulites usually have an area of red, swollen, tender, warm, and is usually caused by *Streptococcus spp.* or *Staphylococcus spp.* (Bjornsdottir *et al.,* 2005; Roberts and Chambers, 2005).

1.2.8.2- Fungi skin infections

Approximately 90% of fungal skin infections are caused by 'dermatophytes', which are parasitic fungi affecting the skin, hair, or nails (John, 1996). There are three groups of dermatophytes, called Trichophyton (affects skin, hair and nails), Microsporum (a type of fungus that causes ringworm epidemics in children) and Epidermophyton (A fungal which grows on the outer layer of the skin and is the cause of tinea). These infections are mostly seen after puberty with the exception of Tinea capitis, which is a fungal infection involving scalp hair, seen in children (Muller *et al.,* 1989).

Other skin infections are caused by yeasts such as Candida. Another known as Malassezia furfur is a type of fungus that causes brownish patches on the skin. This particular yeast resides on skin that has high (oily) sebum content such as the face, scalp and chest. Infection of the skin by *C. albicans* occurs principally in moist, warm parts of the body, such as the axilla, intergluteal folds, and groin or inframammary folds (Kovacs and Hruza, 1995). It is most common in obese and diabetic individuals. These areas become red and weeping and may develop vesicles (John *et al*., 1996).

1.2.9- Methotrexate (MTX)

Methotrexate originated in the 1940 when Dr. Sidney Farber at Children's Hospital Boston was testing the effects of folic acid on acute leukemic children. In the 1948, clinical improvement was observed in these patients who opened avenues in cancer chemotherapy in modern medicine. It gained Food and Drug Administration (FDA) approval as an oncology drug in 1953 (Klareskog *et al.,* 2004).

MTX, formerly known as amethopterin, is classified as an antimetabolite drug which means it is capable of blocking the metabolism of cells. Its brand names: Amethopterin, Immunex, Rheumatrex, Trexal, and Folex PFS (Jay and Ogbru, 2008).

Mejumdor and Aggarwal, (2001), the drug is used to treat certain types of cancer of the breast, skin, head and neck, or lungs. It is also used for treating ankylosing spondylitis, psoriasis, psoriatic arthritis, and rheumatoid arthritis. It has been shown to be effective in inducing miscarriage in patients with ectopic pregnancy. It has a great toxic effect on rapidly dividing cells such as: malignant, myeloid cells, gastrointestinal [GI] and oral mucosa, which replicate their DNA more frequently, and thus inhibits the growth and proliferation of non-cancerous cells as well causing side effects including anemia, neutropenia, increased risk of bruising, nausea and vomiting, dermatitis and diarrhea. A small percentage of patients develop hepatitis, and there is an increased risk of pulmonary fibrosis (Mattes, 1994). Folic acid supplements may help prevent some of these minor side effects (Robin *et al*., 2006).

MTX is taken up by cells and tissues, and converted to MTX-polyglutamtes, long-lived derivatives that retain biochemical and biological activity within the cell. MTX-polyglutamtes can competitively inhibit dihydrofolate reductase

(DHFR), which ultimately affects both purine and pyrimidine nucleotide biosynthesis. Thus during the treatment of cancer with MTX, the malignant cells become starved for purine and pyrimidines, precursors of DNA and RNA require for proliferation (Serra *et al.,* 2004).

Resistance to MTX may occur due to certain mechanisms, including increase in DHFR enzyme level (due to DHFR gene amplification), or due to the impaired intracellular transport of MTX (Kundig *et al.,* 1998; Serra *et al.,* 2004).

Recently, many clinical and pharmacologic studies have confirmed that extracts of Ginger (*Zingiber officinale*) can be helpful in alleviating nausea and vomiting caused by MTX, and also German chamomile (*Matricaria recutita*) has shown to reduce the incidence of mouth sores in people receiving radiation and systemic chemotherapy treatment (Carl and Emrich, 1991; Meyer *et al.,* 1995, Jay and Ogbru, 2008).

1.2.10 - Pathogenesty of studied microorganisms

1.2.10.1- *Staphylococcus aureus*

Staphylococcus is a group of bacteria, familiarly known as *Staph*. These are Gram positive cocci, non-motile, non-spore forming, and facultative anaerobic bacteria (Jawetz *et al.,* 1998). It can be found normally in the nose and on the skin of (20-30) % of healthy adults**,** that can cause serious infections such as infections of the skin can progress to impetigo (a crusting of the skin) or cellulites (inflammation of the connective tissue under the skin, leading to swelling and redness of the area), and a serious complication known as scalded

skin syndrome develops. It causes mastitis (inflammation of the breast) osteomyelitis, bloodstream infections (sepsis), and pneumonia (Stoppler *et al.,* 2007).

1.2.10.2- *Pseudomonas aeruginosa*

The bacteria *P. aeruginosa* is a large genus of Gram-negative, aerobic rod, belonging to the bacterial family *Pseudomonadaceae.* It is found on the skin of some healthy persons and inhabitant of soil, water, and vegetation (Todar, 2004). It is pothogenic only when introduced into areas devoid of normal defenses such as disruption of skin and mucous membrane after direct tissue damage. The bacterium attaches to and colonizes the mucous membranes or skin, invades locally and produces systemic disease. The most serious infections caused by *P. aeruginosa* include infection of wound and burns, malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia, and septicemia (Bodey *et al*., 2007).

1.2.10.3- *Escherichia coli*

E. coli is a Gram-negative bacteria, motile, rods, facultative anaerobic, related to Enterobacteriaceae family. It is a member of the normal intestinal flora and also found as normal flora of upper respiratory and genital tracts. It is also associated with urinary tract infection, intestinal disease and some time food poisoning (Humphery *et al*., 1997).

1.2.10.4 - *Klebsiella pneumoniae*

The bacterium is Gram negative rods, non-motile, aerobic and facultatively anaerobic, and encapsulated. It is found in the normal flora of the mouth, skin,

and intestine (Ryan and Ray, 2004). It causes a wide range of diseases, such as pneumonia, urinary tract infections, septicemia, Ankylosing spondylitis, and soft tissue infections (Podschum and Ullmann, 1998).

1.2.10.5- *Candida albicans*

The fungus 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). It is a component of the normal skin flora and also the main cause of mucocutanous fungal diseases in humans (Hay, 1993). Superficial infections by *C. albicans* are common in immunocompromised patients (Schaller *et al*., 2001).

Chapter two

Materials and Methods

2. Materials and Methods

2.1- Materials

2.1.1- Equipments and instruments

Table 1: The following equipments and instruments were used throughout this project.

Equipment	Company and Origin
Autoclave	Karl (Germany)
Centrifuge	Eppendrorf (Germany)
Clevenger-type distillatory	Halzfeld (Germany)
Coffee grinder	Moulinet (China)
Distillatory unit	Karl (Germany)
Electric Oven	Gallenkamp (England)
Heater	Gallenkamp (England)
Incubator	Sanyo (Japan)
pH-meter	Mettler (England)
Refrigerator	Ishtar (Iraqi)
Sensitive balance	Delta Range (Switzerland)
Soxhelt	Electrothermal (England)
U.V-visible Spectrophotometer	Shimadzu (Japan)

Water bath	Gallenkamp (England)
Instrument	
Filter papers	Halzfeld (Germany)
Glass flasks	Terumo (Japan)
Glass pipettes	Halzfeld (Germany)
Instrument	**Company and Origin**
Gas burner	Grade (England)
Loop	Grade (England)
Micropipette (Different sizes)	Eppendorff (Germany)
Pasteur pipettes	Brand (Germany)
Petridishes	Sterilin (England)
Tips (Different sizes)	Jippo (Japan)

2.1.2- Chemicals

Table 2: The following chemicals were used throughout this project.

Chemical	Company and Origin
Absolute methanol	BDH (England)
Acetic anhydride	BDH (England)
Chloroform	BDH (England)
Ethanol 96%	BDH (England)
Ferric chloride	BDH (England)
Hydrochloric acid (HCL)	Fluka (Switzerland)
Mercuric chloride	BDH (England)

Methotrexate (MTX)	Unterach (Austria)
potassium iodide	BDH (England)
Petroleum ether	BDH (England)
Potasium dihydrogen phosphate (KH_2PO_4)	Fluka (Switzerland)
Chemical	**Company and Origin**
Sodium sulphate (anhydrous)	BDH (England)
Tryptone	Makhur (Germany)

2.1.3- Culture Media

Table 3: The following media were used in this study:

Medium	Company and Origin
Nutrient broth	Oxide (England)
Tryptone soya agar	Oxide
Modified Sabouraud Agar	Oxide

2.1.4- Collection of plant material

Tagetes plant, *T. patula* (Qadifah) flowers were collected from local public gardens in Baghdad city. Plants were identified by Prof. Dr. Ali Al- Mousawy, college of science, Baghdad University. Flowers were air dried at room temperature and grinned into powder by using coffee grinder.

2.1.5- Skin ointment (Samacycline)

In this study, skin ointment (Samacycline) considered as antibacterial ointment was used to treat the infected area. This ointment was used for comparison with the effect of essential oil and methanolic flowers extract. It is manufactured by the state company for drug industries and medical appliances, Samarra, Iraq (Samacycline ointment contains tetracycline HCI 3%).

2.1.6- Microorganism isolates

The microorganisms used are: *S. aureus*, *P. aeruginosa*, *E. coli, K. pneumoniae* (Bacteria), and *C. albicans* (Yeast). These microorganisms were obtained from the Department of Biotechnology, Al-Nahrain University.

The bacterial system (G-system) in this study also was isolated in Institute of Genetic Engineering and Biotechnology for Postgraduate Studies (IGEBPS). The adopted system was consisted of three isolates *Bacillus spp.* (G_3), *Arthrobacter spp.* (G_{12}), and *Brevibacterium spp.* (G_{27}).

Bacteria were grown in the nutrient broth at 37°C for 24 hrs and maintained on tryptone soya agar slants at 4°C.While fungi *Candida* was subcultured into fresh modified Sabouraud agar slants and stored at room temperature.

2.1.7- Laboratory animals

Healthy female BALB/C mice with age range (6-8) weeks weighting (20-30) g were obtained from the Biotechnology Research Center, Al-Nahrain University. Animals were kept in six small plastic cages {29x12.5x11.5} cm, each was containing 3 females. 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). Floors of the cages were covered with soft crushed wood shavings. Animals were kept under suitable environmental

conditions at (26-28°C) and 14 hr day-light daily. Tap water and pellet were accessible freely for mice (Vodopich and Moore, 1992).

2.2- Methods

2.2.1-Preparation of plant extracts

2.2.1.1-Extraction of essential oil

A quantity of 50g of the dried flowers was placed in 500ml D.W., placed in Clevenger apparatus for 3hrs, the essential oil was separated. The oil was dried over anhydrous sodium sulphate and stored at 4°C (Rai *et al.,* 2002).

2.2.1.2- Extraction with methanol

A quantity of 50g of flower powder was mixed with 250ml of 70% methanol by soxhlet apparatus for 6 hrs. at 70°C, then the solvent was removed under reduced pressure by rotary evaporator at 40°C. The crud solid extracts were kept in a freezer until use (Saleem *et al.,* 2004).

2.2.2-Preparation of reagents and solutions

2.2.2.1- Reagents

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

2.2.2.1.1- Mayer's reagent

Two stock solutions were prepared:-

Solution A: A quantity of 35g of mercuric chloride was dissolved in 60 ml of D.W.

Solution B: A quantity of 5g of potassium iodide was dissolved in 10 ml of D.W., solutions A and B were mixed and then the volume was completed to 100ml with D.W. (Smolenski *et al*., 1972).

This reagent was used for the detection of alkaloids.

2.2.2.2- Solutions

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

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

• Mercury chloride solution 1 % (w/v): Prepared by dissolving 1 g of mercuric chloride in 100 ml of D.W. It is used for saponins detection.

2.2.2.3- Diluted solutions

This was prepared by dissolving 0.1g of tryptone powder in 100ml of D.W., then used for diluting microorganisms culture.

2.2.2.4- Phosphate Buffer Saline (PBS)

The PBS was prepared by dissolving 0.86g of KH_2PO_4 in 100ml of D.W., mixed well. The pH was adjusted to 5.5 with 1N HCL. The solution was dispensed in sterile tubes; each was containing 5ml, then sterilized by autoclaving. The stock solution was stored at -4°C (Hudson and Hay, 1980).

2.2.2.5- Methotrexate

It was prepared by mixing 0.2ml of MTX with a concentration of 25mg/1ml, in 4.8ml D.W. under aseptic conditions, with final concentration of 1000µg/ml, and kept in the refrigerator. Aliquot of 0.25ml of the prepared concentration was added to 5ml of BPS in order to obtain a final concentration of 50µg/ml (Al-Shimary, 2000).

2.2.3- Detection of some active compounds in plant extracts

Several tests were implemented to detect some active compounds in plant extracts.

2.2.3.1- Detection of phenols

Aliquot of 2ml of aqueous ferric chloride 1% was mixed with 2 ml potassium iron cyanide 1%. Equal quantity of reagent and plant extract was used. The appearance of blue-green color indicated the presence of phenols (Harborne, 1984).

2.2.3.2- Detection of flavonoids

Flowers methanol extract was partitioned (Separatory funnel) with petroleum ether, the aqueous layer was mixed with the ammonia solution. The appearance of dark color was an indication for the presence of flavonoids (Harhorne, 1984).

2.2.3.3 - Detection of alkaloids

Aliquot of 0.2ml of the methanol extract was added to 5ml of 1% HCL using a steam bath, and then 1ml of the filtrate was treated with Mayer's reagent. The appearance of white precipitate was an evidence for the presence of alkaloids (Treas and Evans, 1987).

2.2.3.4- Detection of terpens

A quantity of 1g of the dried methanol extract was suspended with a few drops of chloroform, and then a drop of acetic anhydride and a drop of concentrated H_2SO_4 were added. Appearance of brown color indicated the presence of terpenes (Al-Abid, 1985).

2.2.3.5- Detection of saponins

Aliquot of 5ml of the flowers extract was added to (1-3) ml of mercury chloride solution. A white precipitate was developed indicating the presence of saponins (Stahl, 1969).

2.2.4- Measurement of the acidity in extracts

Aliquot of 10 g of flowers powder was mixed with 250ml of 70% methanol for 10 min using magnetic stirrer. The suspension was filtered and the acidity of the filtrate was measured with pH-meter (Shihata, 1951).

2.2.5- Preparation of culture media

The following culture media were used in this project

A- **Tryptone soya agar**

It was prepared by mixing 37g of tryptone soya agar with 1L D.W., then the culture media were sterilized by autoclaving at 121°C for 15 min under pressure of 1.04 $kg.cm^{-2}$(Cappaueecino and Sharmany, 1987).

B- **Nutrient broth**

A quantity of 3.7g of nutrient broth powder was dissolved with 100ml of D.W., them sterilized by autoclaving at 121°C for 15 min.

C- Preparation of Modified Sabouraud Agar:

C. albicans 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).

2.2.6- Sterilization methods

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

B- Plant extracts

The prepared stock solutions of flower extracts were sterilized by using Millipore filter unit with pore size of 0.45µm. (Adeeba, 1982).

2.2.7- Determination of antimicrobial activity (*in vitro*)

Essential oil was taken at concentrations (250, 500, and 750) ppm and undiluted concentration. These concentrations were prepared according to the equation:

$$ppm = \frac{X \quad 1000}{\text{Media size}}$$

(X: represents the volume of used extract).

The stock solutions of methanol extract to be used as antimicrobial agent were prepared by dissolving 1 g of each plant extract residue with 10ml 70% methanol (Jawad *et al.,* 1988). The stock solution was sterilized by ultrafiltration using Milipore filter 0.45µm under aseptic conditions (Ibrahim, 2003). Different concentrations (75, 50, 25 and 12.5) mg/ml of the flower extract.

The nutrient agar medium was mixed well and 20 ml was poured into Petri-dishes. The surface of the medium was swabbed with 0.1 ml of a suspension containing 1.5×10^8cfu/ml of the pathogenic bacteria (*S. aureus*, *P. aeruginosa*, *E. coli,* and *K. pneumoniae*) and 1.5×10^3cfu/ml of the pathogenic

yeast (*C. albicans*) using sterile cotton swab. The hole-plate diffusion technique was used. Five plugs were removed from each agar plate using a sterile cork borer to make 5mm diameter holes. To each hole, 100µl from different concentrations of each extract was added and allowed to diffuse at room temperature for 20 min (Shihchih *et al.,* 1990; Essawi and Srour, 2000; El- Astal *et al.,* 2005).

A control well was made in the center of the plate's swapped with 0.01 ml of the extracting solvent diluted with D.W. or methanol absolute. The inoculated plates were incubated at 37ºC for 24 hrs. (Jawad *et al.,* 1988; NCCLs, 2005).

2.2.8- Administration of extracts on laboratory animals

2.2.8.1- Experimental animals (*in vivo*)

Six groups of mice were used. They were divided as follows:

- First group (A) negative control: Infected with *S. aureus* then treated with D.W. (3 mice).
- Second group (B) positive control: Infected with *S. aureus* then treated with Samacycline ointment (3 mice).
- Third group (C): Infected with *S. aureus* then treated with methanol extract of 50mg/ml (3 mice).
- Fourth group (D): Infected with a sample prepared 24 hrs. before used containing *S. aureus* and methanol extract of 50mg/ml (3 mice).
- Fifth group (E): Infected with *S. aureus* then treated with essential oil (3 mice).
- Sixth group (F): Infected with a sample prepared 24 hrs. before used containing *S. aureus* and essential oil (3 mice).

2.2.8.2- Treatment of animals

Skin of the mice's back was shaved using a razor for hair removal, cleaned and disinfected with cotton swabbed in saturated 70% alcohol. The skin of each mouse was scraped by using pathological scalpel to make abrasion, after half an hour, a micropipette was used to smear 0.1ml of the bacteria suspensions with *S. aureus* on the abraded area.

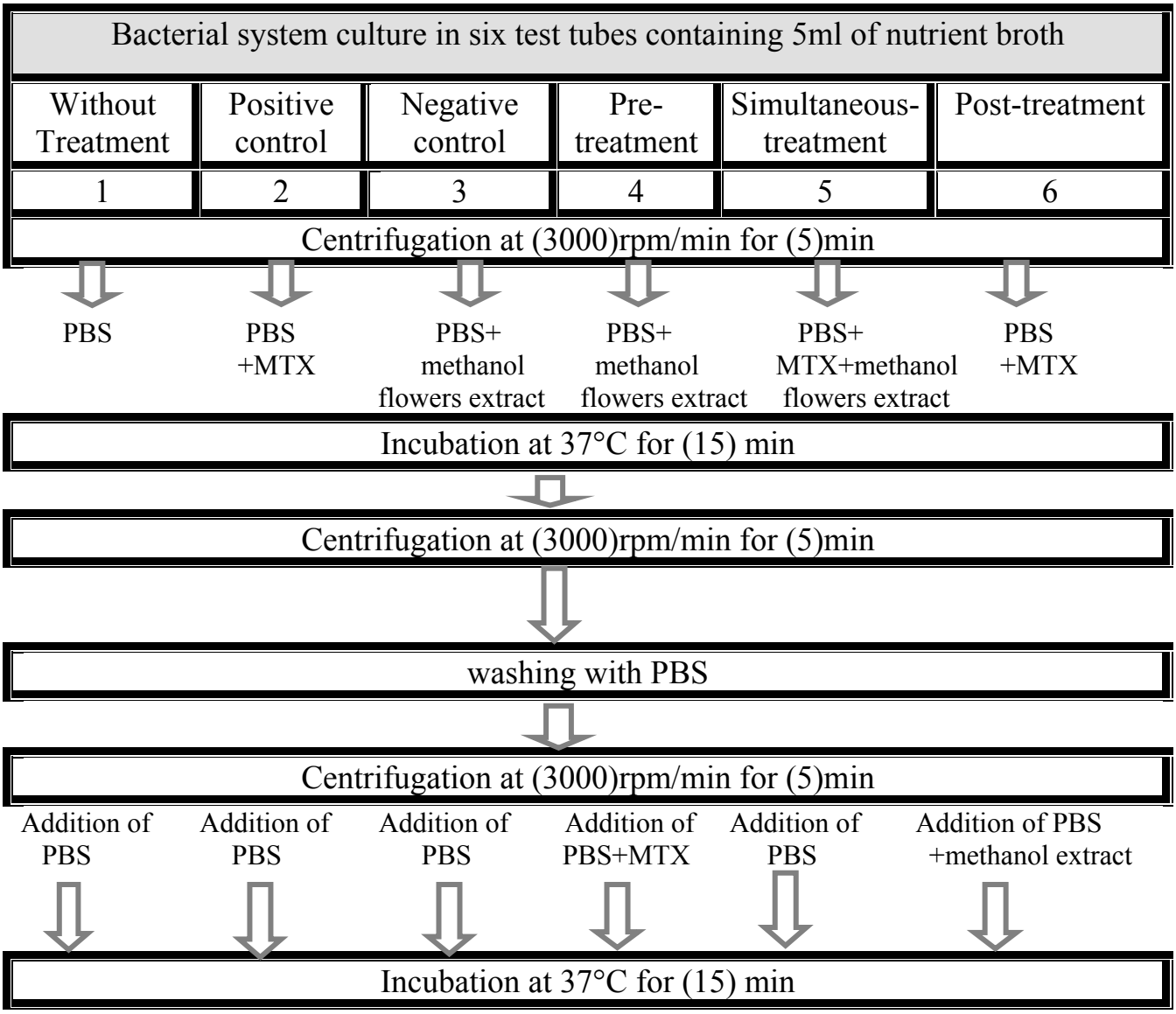
Group A (Negative control) was treated topically with D.W. that applied on either sides of shaved surfaces after 3 days of infection when the lesions were exerted. Group B (Positive control) was treated topically with commercial skin ointment Samacycline. While group C was treated topically with flowers methanol extract (50mg/ml). Group D was treated topically with a sample prepared as follows: aliquot of 0.5ml of methanol extract (50mg/ml) was mixed with 0.5ml of nutrient broth medium, and then 0.1ml of *S. aureus* suspensions was inoculated and incubated at 37°C for 24 hrs., there after 50µl droplets of this sample were placed on the abrasion skin. The applied area of the skin was examined daily and compared with control animals. Group E was treated topically with essential oil, and group F was treated topically with a sample prepared as follows: aliquot of 0.5ml of essential oil was mixed with 0.5ml of nutrient broth medium, and then 0.1ml of *S. aureus* suspensions was inoculated and incubated at 37°C for 24 hrs., then 50µl droplets of this sample were placed on the abrasion skin. The therapeutic effect of plant extract was studied and compared with that of Samacycline ointment.

2.2.9-The interaction between plant extract and MTX (*in vitro*)

The steps of conducted the experiments were explained in Figure 2.

Prepared the bacterial culture logarithmic for all bacterial system (G-system) in six test tubes containing 5ml of nutrient broth whoever isolation (Al-Shimary, 2000).

Cells were separated by centrifugation at 3000rpm/min for 5min, and them washed with 3ml of PBS (pH=5.5). After washing, the cells were inoculated into test tubes containing 5ml of PBS.



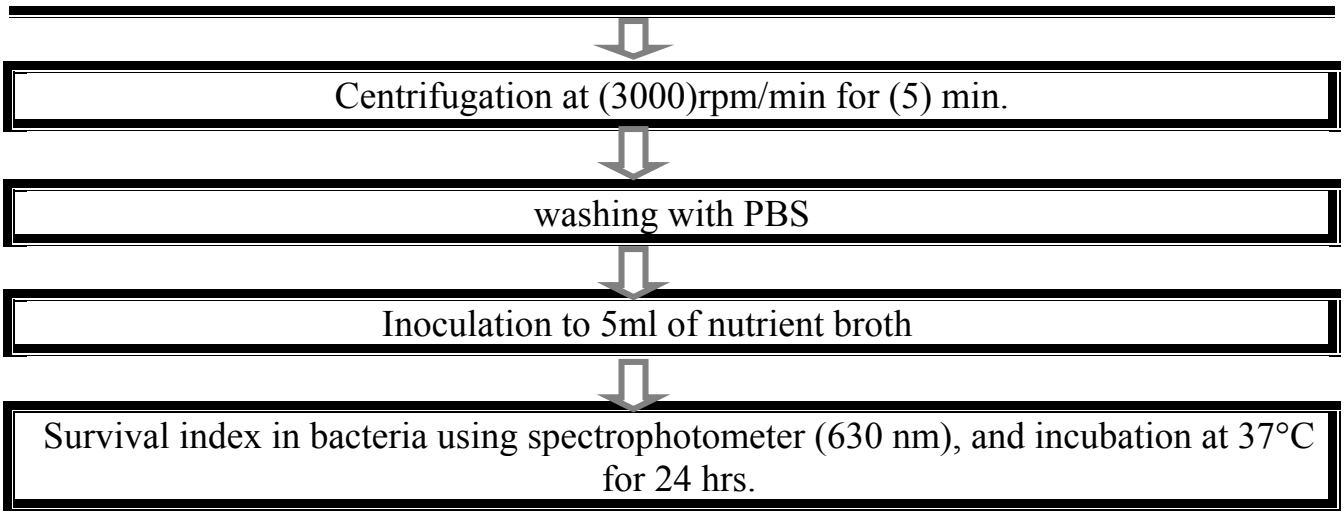


Figure 2: The procedures followed in conducting experiments.

A- First treatment (Negative control)

1- Test tubes containing cells were transferred for centrifugation at (3000) rpm/min for 5 min.

2- Incubation of the test tubes at 37°C for 15 min.

3- Cells were washed with 3ml of PBS, and then centrifuged at (3000) rpm/min for 5 min.

4- Test tubes containing 5ml of nutrient broth were incubated. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630 nm.

5- Test tubes were incubated at 37°C for 24 hrs.

B- Second treatment (Positive control)

1- Addition of MTX (1ml) at a concentration of 50µg/ml into test tubes containing 5ml of PBS and cells.

2- Incubation of the test tubes at 37°C for 15 min.

3- Centrifugation at 3000 rpm/min for 5 min.

4- Cells were washed with 3ml of PBS, and then centrifuged at 3000rpm/min for 5 min.

5- Test tubes containing 5ml of nutrient broth were incubated. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630 nm.

6- Test tubes were incubated at 37°C for 24 hrs.

C- Third treatment

1- Addition of methanol flower extracts (1ml) at a concentration of 50 mg/ml into test tubes containing 5ml of PBS and cells.

2- Incubation of the test tubes at 37°C for 15 min, then centrifuged at 3000rpm/min for 5 min.

3- Cells were washed with 3ml of PBS, and then centrifuged at 3000rpm/min for 5 min.

4- Test tubes containing 5ml of nutrient broth were incubated. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630 nm.

5- Test tubes were incubated at 37°C for 24hrs.

D- Fourth treatment (Pre-treatment)

1- Addition of methanol flower extracts (1ml) at a concentration of 50mg/ml into test tubes containing 5ml of PBS and cells.

2- Incubation of the test tubes at 37°C for 15 min, then centrifuged at 3000 rpm/min for 5 min.

3- Cells were washed with 3ml of PBS, and then centrifuged at 3000 rpm/min for 5 min.

4- Cells were inoculated into test tubes containing 5ml of PBS.

5- Addition of 1ml of MTX into the test tubes.

6- Incubation the test tubes at 37°C for 15 min, then centrifuged at 3000rpm/min for 5 min.

7- Cells were washed with 3ml of PBS, and then centrifuged at 3000 rpm/min for 5 min.

8- Test tubes containing 5ml of nutrient broth were incubated. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630nm.

9- Test tubes were incubated at 37°C for 24hrs.

E- Fifth treatment (Simultaneous-treatment)

1- Addition of (1ml) methanol flower extract and (1ml) of MTX into test tubes containing 5ml of PBS and cells.

2- Incubation of the test tubes at 37°C for 15 min, and then centrifuged at 3000 rpm/min for 5 min.

3- Cells were washed with 3ml of PBS, and then centrifuged at 3000 rpm/min for 5 min.

4- Test tubes containing 5ml of nutrient broth were incubated. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630 nm.

5- Test tubes were incubated at 37°C for 24 hrs.

F- Sixth treatment (Post-treatment)

1- Addition 1ml of MTX at a concentration of 50µg/ml into test tubes containing 5ml of PBS and cells.

2- Incubation of the test tubes at 37°C for 15 min, and centrifuged at 3000 rpm/min for 5 minutes.

3- Cells were washed with 3ml of PBS, and then centrifuged at 3000 rpm/min for 5 minutes.

4- Cells were inoculated into test tubes containing 5ml of PBS.

5- Inoculation of 1ml of methanol flower extract into the test tubes.

6- Incubation of the test tubes at 37°C for 15 min, and centrifugation at 3000 rpm/min for 5 min.

7- Cells were washed with 3ml of PBS, and then centrifuged at 3000 rpm/min for 5 minutes.

8- Test tubes were inculcated with 5ml of nutrient broth. These samples were used for the detection of survival index after treatment using spectrophotometer at wave length 630 nm.

9- Test tubes were incubated at 37°C for 24 hrs.

The survival index was determined in all treatments after 24 hrs. It was calculated according to the following equation (Eckardt and Haynes, 1981):

$$Sx = Ns / No$$

(Sx: survival index, Ns: number of cells after treatment, and No: number of cells in negative control).

This experiment was repeated three times for the three bacterial isolates (G_3, G_{12}, and G_{27}).

2.2.10- Experimental design and statistical analysis

Completely randomized design (CRD) has adopted for experimental design with a number of replicates as indicated a tables and figures.

Analysis of variance (ANOVA) was performed to test whether group variance was significant or not, according to Al-Mohammed *et al.,* (1986).

Chapter three

Results and Discussion

3. Results and Discussion

3.1- Detection of some active compounds

Various active compounds were detected in *T. patula* methanol flower extract as shown in table 4. Results indicated the presence of phenols, flavonoids, alkaloids, terpens, and saponins. Such finding are in accordance with the results of Hadjiakhoondi *et al.,* 2005 who reported that methanol extract of *T. patula* contains alkaloids, phenols, terpens, saponins, and flavonoids. The pH of methanol extract of *T. patula* was 5.4 which represent the acidity.

The beneficial medicinal effects of the extract typically result from the secondary products present in flowers. Existence of secondary metabolites is unique to the particular plant species or families (Wink, 1999).

Table 4: Some secondary metabolites detected in *Tagetes patula* flowers methanol extract

Compounds	Indicator	Result of detection
Phenols	Blue-green color	+

Flavonoids	Dark green color	+
Alkaloids	White precipitate	+
Terpenoids	Brown color	+
Saponins	White precipitate	+

+: indicates the presence of the compound.

3.2- Detection of antimicrobial activity of essential oil and methanol extract (*In vitro*)

3.2.1- Antimicrobial activity of essential oil

The essential oil of *T. patula* showed an inhibitory effect on the growth of bacteria and yeast as shown in table 5 and appendix 1.

Essential oil was more effective against *S. aureus* with little effect against *P. aeruginosa*, and *K. pneumoniae* at undiluted concentration (Appendix 1), whereas it showed no activity against *E. coli*. The growth of *C. albicans* was highly affected after the treatment with undiluted concentration (Appendix 1). Results obtained from this study are in agreement with those of Maria *et al.,* (2006) and Dutta *et al.,* (2007) who reported that *T. patula* essential oil has antimicrobial activity against many microorganisms except *E. coli*. While, Maria *et al.,* (2006) regarded *E. coli* sensitive to essential oil extracted from *T. patula* flowers.

Testing and evaluation of the antimicrobial activity of essential oils is considered difficult because of its volatility, water insolubility and complexity (Janssen *et al*., 1987). The resistance of Gram-negative bacteria could be due to

the permeability barrier provided by cell wall (Adwan and Abu-Hasan, 1998). The *in vitro* antimicrobial activity of the essential oil may be due to terpinol (Dewick, 1998).

Table 5: Growth of Microorganisms under investigation as affected by different concentrations of essential oil extracted from *T. patula* flowers

Concentration of essential oil (ppm)	Diameter of inhibition zone (mm)				
	S. aureus	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C.albicans*
Control (D.W.)	0.00	0.00	0.00	0.00	0.00
250	0.00	0.00	0.00	0.00	0.00
500	0.00	0.00	0.00	0.00	0.00
750	0.00	0.00	0.00	0.00	0.00
undiluted	16 ± 0.5	0.00	Slight inhibition	Slight inhibition	18 ± 0.7

Values are means of five sample readings ±SD

The difference in activity of essential oil against the bacteria and fungi may be due to the differences in constituents of this oil. The essential oil in *T. patula* flowers contains high levels of piperitone, trans-β-ocymene, and β-caryophyllene. According to related studies, those compounds are considered active as bactericides, fungicides, disinfectants, and virucides. It showed high

inhibition activity against *S. aureus*, *P. aeruginosa*, *Salmonella typhi*, *Enterococcus hirae*, *Salmonella choleraesuis*, *Bacillus subtilis*, *Bacillus cereus*, *Acinetobacter calcoaceticus*, *Clostridium sporogenes*, *Clostridium perfringens*, and *Yersinia enterocolitica* (Yong-Suk and Dong-Hwa, 2005).

The antimicrobial activity of the oil explains its folkloric use in the treatment of bronchitis and inflammations of the eyes. Thus, the essential oil of *T. patula* is recommended for pharmaceutical and perfumery purposes (Kirtikar and Basu, 2000).

3.2.2- Antimicrobial activity of methanol extract

Tagetes methanol extract exhibited antimicrobial action against most tested microorganisms at concentrations 50 and 75 mg/ml. The largest inhibition zone was observed against *S. aureus* recording 25mm in diameter at the concentration 50 mg/ml whereas, reduced to 20mm at the concentration 75 mg/ml. At the concentration 25 mg/ml, *S. aureus* showed 14mm followed by *C. albicans* which showed 19 and 15.2 mm inhibition zone at 50 and 75 mg/ml, respectively, and slight inhibition was observed at the concentration 12.5 mg/ml (Table 6 and Appendix 2).

Table 6, shows that *P. aeruginosa* and *K. pneumoniae* gave a diameter of inhibition zone 10.5 and 12mm in the concentration 75 mg/ml, respectively, while reached 13.3 and 17 mm at the concentration 50 mg/ml, respectively. *E. coli* showed no inhibition in all concentrations of the methanol extract.

The antimicrobial activity of *T. patula* flower extract is only detectable at high concentrations (up to 50 mg/ml of the crude extract). Generally, the antimicrobial activity of any extract depends on the type of extract,

concentration and the type of microorganism to be examined (Rauha *et al.,* 2000).

It is clear from the data presented in table 6 that among the five tested microorganisms, *S. aureus* was the most susceptible one to the two types of extracts. The finding that *S. aureus* is susceptible to a variety of extracts was recorded by others (Okemo *et al.,* 2001; Madamombe and Afolayan, 2003). Variations in the susceptibility of bacterial species may be due to the genetic variation, and cellular structure (Mitscher *et al*., 1972; Frazier and Westhoff, 1987). Furthermore, the results are in agreement with Piccaglia *et al.,* (1998) and Guinot *et al.,* (2007) who showed the inhibitory activity of *T. patula* flower methanol extracts against Gram-positive bacteria and Gram-negative bacteria when applied at high concentrations.

Table 6: Growth of microorganisms under investigation as affected by different concentrations of methanol extracts.

Concentration of methanol extract (mg/ml)	Diameter of inhibition zone (mm)				
	S. aureus	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*C. albicans*
Control (methanol absolute)	0.00	0.00	0.00	0.00	0.00
12.5	10 ±0.06	0.00	0.00	0.00	Slight inhibition
25	14 ±0.18	0.00	0.00	Slight inhibition	12 ±0.13
50	25 ±0.88	0.00	13.3 ±0.18	17 ±0.63	19 ±0.88
75	20 ±0.88	0.00	10.5 ±0.06	12 ±0.13	15.2 ±0.20

Values are means of five sample readings ±SD

It appears that methanol extract is more efficient than essential oil; the reason for this may be due to the compounds already extracted by methanol particularly phenolic compounds as stated by Cowan, (1999).

The site and number of hydroxyl groups on the phenol are thought to relate to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Taiz and Zeiger, 2002). In addition, some authors have found that more highly oxidized phenols are inhibitory to growth of microorganisms (Scalbert, 1991). Jawetz *et al*., (1998) described the mechanism thought to be responsible for phenolics toxicity against microorganisms to membrane disruption, binding or adhesion making a complex with cell wall, inactivation of enzymes, and binding to proteins.

Results indicated the abundance of flavonoids, which agreed with Tereschuk *et al.,* 2004 who indicated that *T. patula* is a potential source for flavonoids and therefore it can be used as antimicrobial agent. Flavonoids are also hydroxylated phenolic substances but occur as C6-C3 unit linked to an aromatic ring (Donald and cristobal, 2000). Their activity is probably due to their ability to make complex with bacterial membranes (Cowan, 1999).

Alkaloids detected in *T. patula* are commonly having antimicrobial properties. The effect of alkaloids on microorganisms may be attributed to plasma membrane destruction, the interaction with metabolic reactions, and changes in enzymes vital to growth and reproduction (Abdul- Rahman, 1995).

According to these results, it could be possible to use French marigold essential oil and methanol flower extract as a natural antibacterial and antifungal. This reflects the economic importance of this plant.

3.3- Effect of methanol flower extracts and essential oil on *S. aureus* in experimental animals (*in vivo*)

Certain degenerative changes were noticed after 3 days of infection with *S. aureus* (Figure 3). Red granulated tissue, pus and separation of small sheets of epidermis were clear in the infected area of skin.

These changes may be due to many virulence factors caused by *S. aureus* such as exfoliative toxin which is an exotoxin produced by *S. aureus*, causing blisters in human and animal skin, detachment within the epidermal layer and the loss of keratinocyte cell-cell adhesion in the superficial epidermis (Nishifuji *et al*., 2008).

Figures 4, and 5 exhibit the skin of mouse treated with flower extract showing curing of the infected area, growth of hair however, still showing a mark between regenerative area and normal area. When the above results were compared with the animals that infected with *S. aureus* then treated with D.W. (Group A), after the 8^{th} day of the treatment, slower regenerative changes were seen (Figure 5). Group B treated with commercial Samacycline ointment have shown redness which disappeared at the 4^{th} day of the treatment (Figure 4), but the initial depression of the healing incision lines disappeared at the 8^{th} day of the treatment (Figure 5). While group C that treated with methanol flower extract showed certain regenerative changes. The redness disappeared at the 4^{th} day of the treatment (Figure 4). The initial depression of the healing incision lines disappeared at the 8^{th} day of the treatment (Figure 5). Group E displayed the same regenerative changes but treated with essential oil of *T. patula*. The redness disappeared at the 5^{th} day of the treatment (Figure 4). The initial depression of the healing incision lines disappeared at the 10^{th} day of the treatment (Figure 5). This incision was completely recovered with newly formed epithelium.

Groups D, and F, represent the scratched skin of animals that infected with *S. aureus* and treated with methanol flower extract at 50mg/ml (group D) or infected with *S. aureus* and treated with essential oil (Group F). The infection recovered at the 8th day of the infection (Figure 5), indicating that tagetes methanol and essential oil extracts besides inhibiting the growth of *S. aureus*, it facilitated the healing of infected skin.

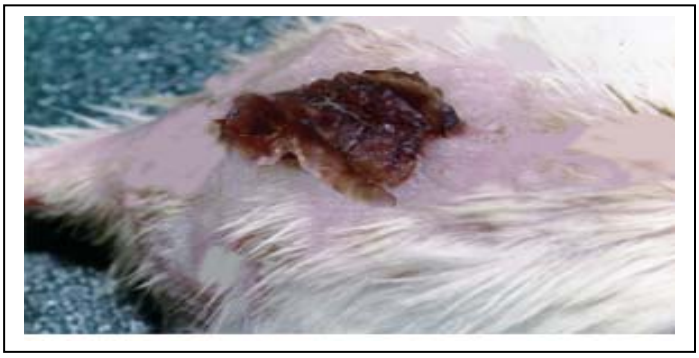
Extracts of *T. patula* flowers were considered to have antibacterial activity, due to the presence of phenolics and Terpenoids compounds (Cowan, 1999), the group which treated with methanol extract, redness was still persist with peeling (Figure 5) indicating that Samacyclin ointment is more effective than methanol flower extract in healing the infected area suspecting that time for healing after treatment with essential oil was less than that treated with methanol extract which is also another fact for the activity of the extracts.

Three general modes of action for different plant extracts were recognized by Tayler *et al*., (1999) as follows:

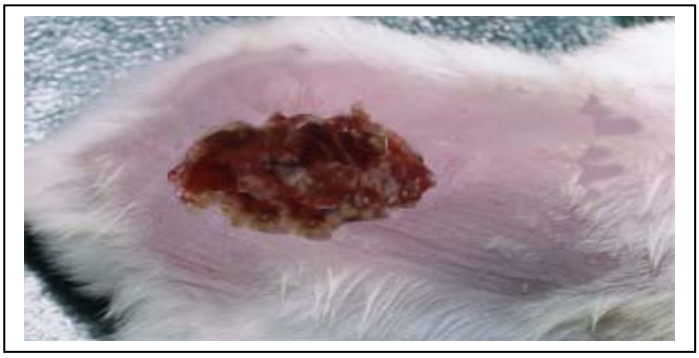
a- Inhibition of the microbial cell wall formation or biosynthesis of some essential proteins.

b- Disruption of deoxyribonucleic acid (DNA) metabolism.

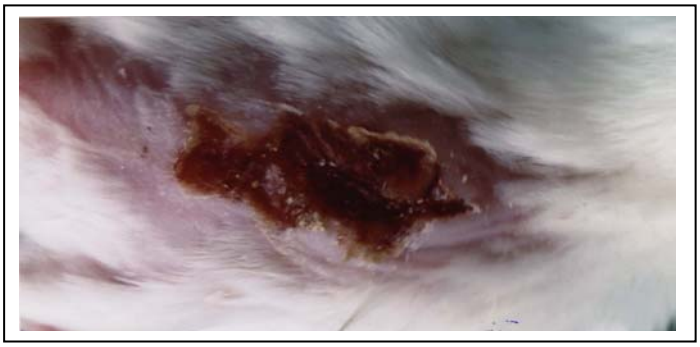
c- Alteration of normal function of cellular membrane.



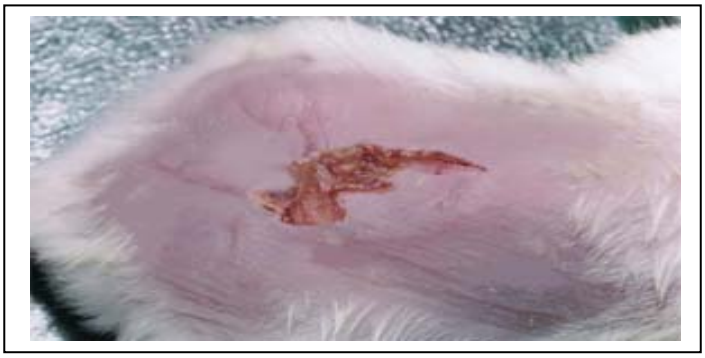
A



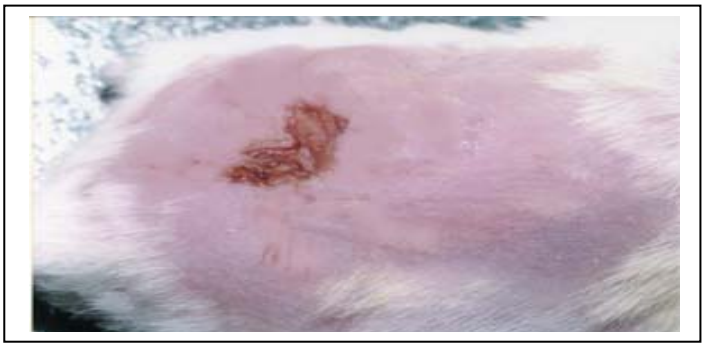
B



C

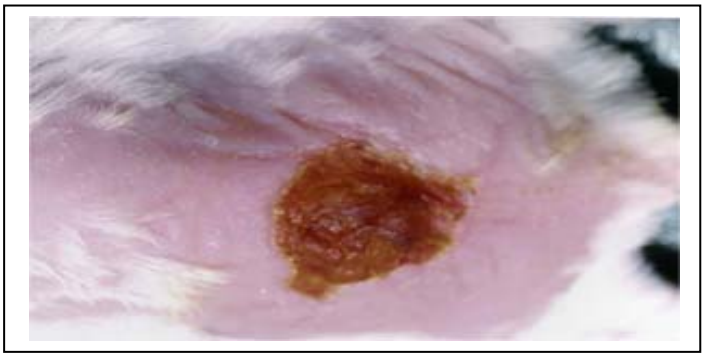


D

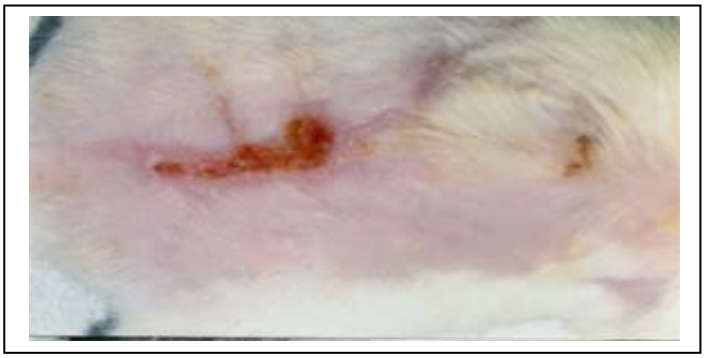


E **F**

Figure (3): (A, B, C, and E) morphological changes in animal's skin caused by *S. aureus*, (D) morphological changes caused by (*S. aureus* and methanol extract), and (F) morphological changes caused by the sample (*S. aureus* and essential oil) at 3rd day

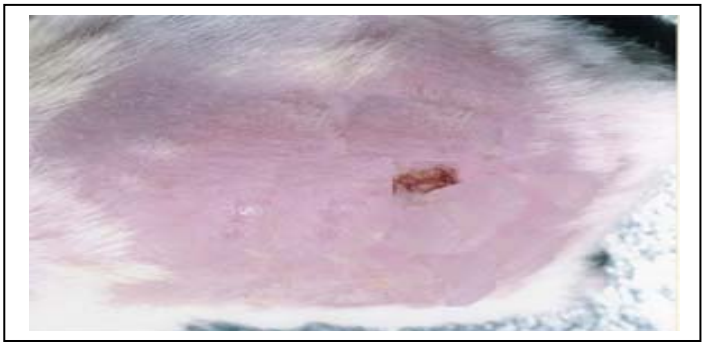


A

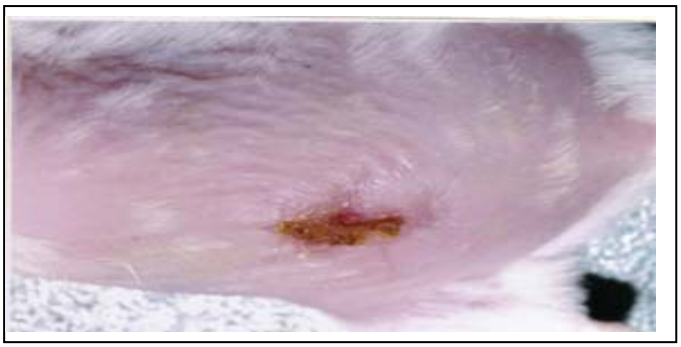


B

C



D

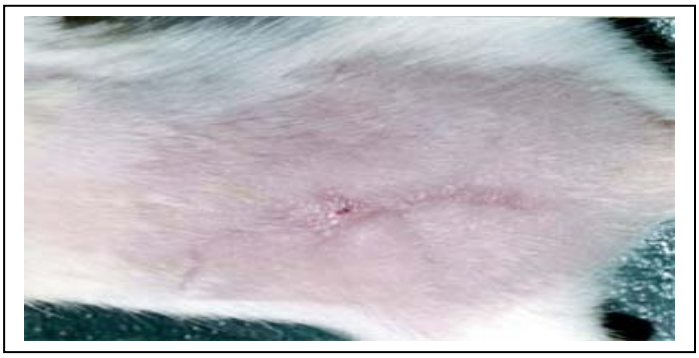


E **F**

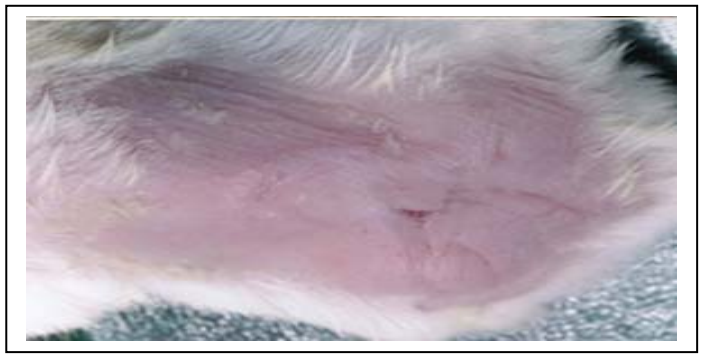
Figure (4): Morphological repair of the skin infected by *S. aureus* and treated with D.W. (A), Samacyclin (B), methanol extract (C), *S. aureus* and methanol extract (D), essential oil (E), and *S. aureus* and essential oil (F) at 4th day of treatment.

A

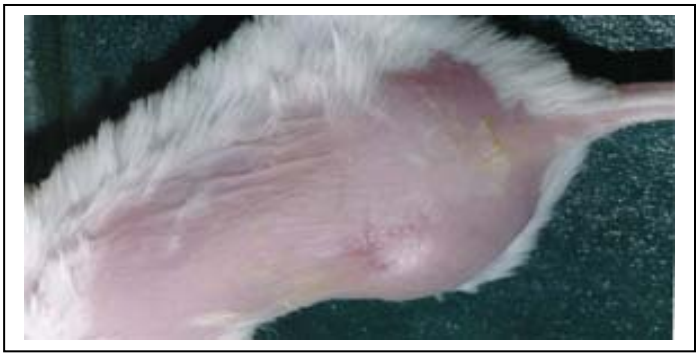
B



C



D



E **F**

Figure (5): Morphological repair of the skin infected by *S. aureus* and treated with D.W. (A), Samacyclin (B), methanol extract (C), *S. aureus* and methanol extract (D), essential oil (E), and *S. aureus* and essential oil (F) at 8th day of treatment.

3.4- Interactions between plant extract and MTX (*in vitro*)

The concentration 50µg/ml of MTX was used as a positive control, and this concentration led to a significant reduction ($p \leq 0.05$) in survival index for the three isolates of G- system (G_3, G_{12}, and G_{27}) as compared to G- system treated with PBS (Negative control). Survival index of (pre, simu, and post) treatments with MTX behaved in a similar manner for the three isolates of G- system. Interactions between methanol extract at a concentration 50mg/ml and MTX for G3 (0.61, 1.00, 1.04), G_{12} (0.86, 0.96, 1.03), and G_{27} (0.86, 1.00, 1.03) were significantly higher than positive control (Figure 6, 7, 8, and Appendices 3, 4, 5).

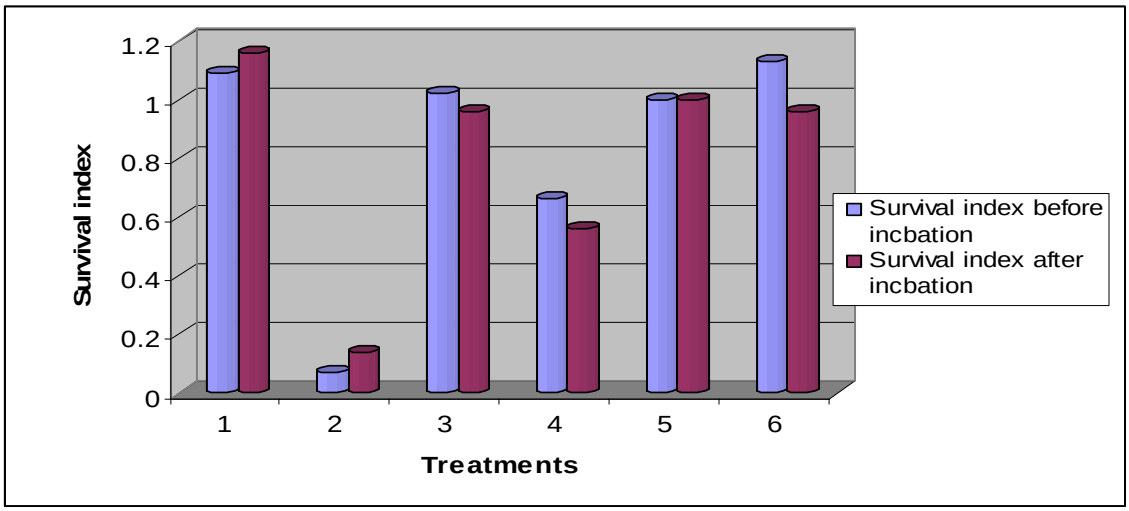


Figure 6: Interaction effect between methanol extract of *T. patula* and MTX on survival index for bacteria G3 before and after incubation:
1. Negative control, 2. Positive control with MTX only, 3. Treatment with flower extract, 4. Treatment with flower extract before treatment with MTX, 5. Treatment with a combination of flower extract and MTX, 6. Treatment with flower extract after MTX treatment.

The survival index of G- system (G3, G12, and G27) after 24hrs. incubation behaved in a similar manner as before incubation (0.82, 0.79), (0.92, 0.91), and (0.89, 0.87) respectively (Figure 6, 7, 8). Al-Shimary (2000) also found a decreasing effect of MTX in cultured G-system treated with the extract of flax seed (*Linum usitatissimum*).

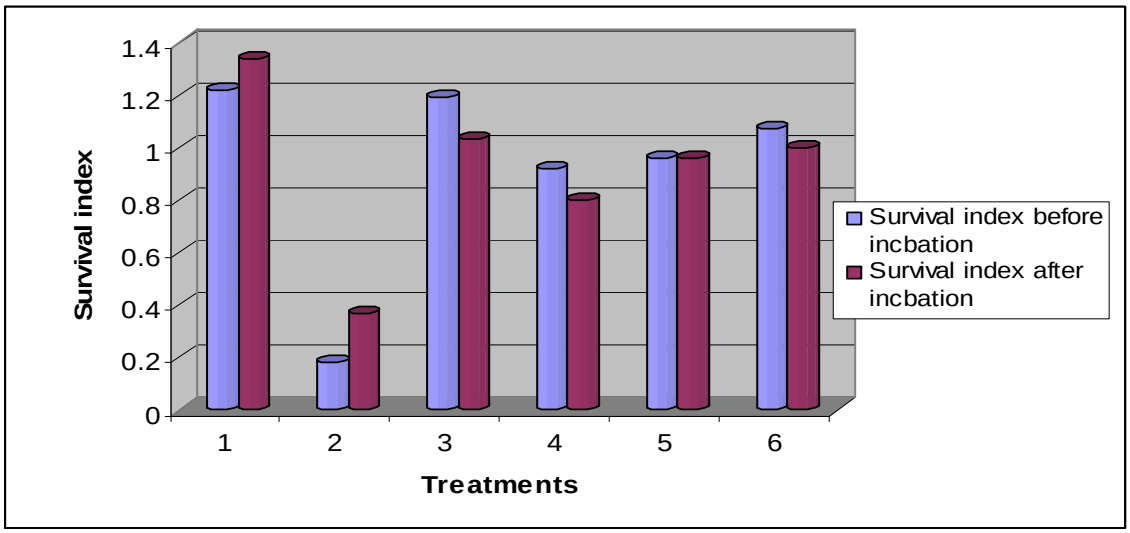


Figure 7: Interaction effect between methanol extract of *T. patula* and MTX on survival index for bacteria G12 before and after incubation:
1. Negative control, 2. Positive control with MTX only, 3. Treatment with flower extract, 4. Treatment with flower extract before treatment with MTX, 5. Treatment with a combination of flower extract and MTX, 6. Treatment with flower extract after MTX treatment.

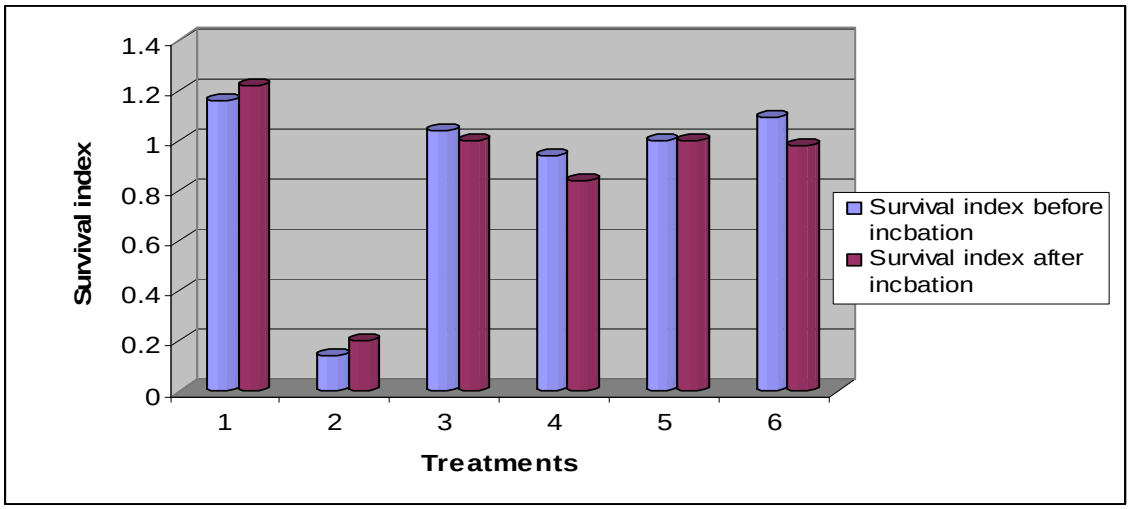


Figure 8: Interaction effect between methanol extract of *T. patula* and MTX on survival index for bacteria G27 before and after incubation:
1. Negative control, 2. Positive control with MTX only, 3.Treatment with flower extract, 4.Treatment with flower extract before treatment with MTX, 5.Treatment with a combination of flower extract and MTX, 6. Treatment with flower extract after MTX treatment.

From the biological perspective, antioxidants provide protection to living organisms from damage caused by uncontrolled production of free radicals / reactive oxygen species (ROS) and concomitant lipid per oxidation, protein denaturing and DNA-strand breaking (Yadav *et al.,* 2003).

The recognized dietary antioxidants are vitamin C, vitamin E, and carotenoids whish are ubiquitous in the plant kingdom, and it has become clear that certain polyphenolics in plants have considerable antioxidant ability *in vitro*, such as scavenging peroxyl radicals, superoxide, ozone, and nitric oxide (Murakami *et al.,* 1994; Gann *et al.,* 1999). In a similar manner to vitamin E, this activity is essential due to the ease with which an H atom from an aromatic hydroxyl (OH) group can be donated to a free radical and the ability of an

aromatic compound to support and unpaired electron due to delocalization around the л-electron system (Duthie and Crozier, 2000).

The significant decrease in MTX toxicity during the frequency of interaction with the plant extract perhaps due to the antioxidant activity of the flower extract. Furthermore, the present results are in accordance with Jian *et al.,* 2007 who have reported that alcohol extracts of dried tagetes flowers contain antioxidant products.

Qadifah (*Tagetes patula*) was found to possess a significant antimicrobial property mainly because it contains a large number of secondary metabolites shown in table 5. Flavonoids and phenolics constituents of tagetes have also been reported to exhibit antioxidant properties. Alpha-tocopherol (vitamin E) and flavonoids contain chemical structural elements that may be responsible for antioxidant activities (Hollman and Katan, 1999). A study by Wenying *et al.,* 2003 suggested that flavonoids can replace vitamin E as chain-breaking antioxidants in liver microsomal membranes.

Numerous studies have reported flavonoid-mediated antiproliferative effects against leukemia, intestinal, lung, breast, and bladder cancer cells (Garry *et al.,* 2000). Flavonoids suppress human leukemic HL60 cell proliferation. Also it is inhibitory to conventional anticancer agents such as doxorubicin and methotrexate (Hirano *et al.,* 1994). The interaction between the antioxidant and chemotherapy appears to be more complex than might be predicted solely on the basis of oxidative mechanisms (Chinery, 1997).

Conclusions and Recommendations

Conclusions

1- Inhibitory effects of essential oil against tested microorganisms in concentration depended except *E. coli* which showed moderate response.
2- Methanol extract of tagetes is more effective than essential oil.
3- Methanol flowers extract did not inhibit toxic effects on the three bacterial isolates (G-system) at the concentration 50mg/ml.
4- Essential oil and methanolic extract are a potential cure for skin infection in test animals compared to the commercial skin ointment (Samacyclin).
5- Methanol flowers extract offered protect on to the three bacterial isolates against the toxic effect of MTX, when was used (pre, with, post) MTX.

Recommendations

1- Further studies are recommended to explore the detailed analysis and purification of chemical composition, and the mode of action for each compound.
2- Examination of different pharmacological as antivirus, anticancer, and insecticidal effect of tagetes active compounds.
3- Exploitation of plant tissue culture technique, for the possible increase in yield of secondary metabolites in callus and cell suspension cultures.
4- Further studies are required to investigate the possible application of tagetes essential oil for food preservation, since it showed anti-microbial activity.
5- Further studies are required to characterize the antioxidant properties of *T. patula* active compounds.
6- Study the interactions of *T. patula* essential oil and extracts with other types of drugs which are commonly used in the treatment of cancers.

References

References

Abdul-Rahman, G. Y. (1995). Effect of some medical plants and chemicals on the growth of pathogenic bacteria. J. Vet. Sci., 8(20): 101-108.

Abuharfeil, N. M.; Maraqa, A. and Vonkleist, S. (2000). Augmentation of natural killer cell activity *in vitro* against tumor cells by wild plants from Jordan. J. Ethnopharmacol., 71: 55-63.

Adeeba, S. Y. (1982). The molecular effect of some plant extracts on the growth and metabolism of some Gram-negative and Gram-positive bacteria, M.Sc. thesis, Dept. of Microbiology, College of Science, University of Babylon, Iraq.

Adwan, K. and Abu-Hasan, N. (1998). Gentamicin resistance in clinical strains of Enterobacteriaceae associated with reduced gentamicin uptake. Folia. Microbiol., 43: 438-440.

Al-Abid, M. (1985). Zur zusammem setrung der absuchlu bmemtrame in phoenix dactily frawuzburg. Univ. Wurzburg F. R. of Germany. Planta, 67: 260-274.

Al-Mohammed, N. T.; Al-Rawi, K. M.; Younis, M. A. and Al-Morani, W. K. (1986). Principles of Statistics. Al-Mosil University Press, Iraq (in Arabic).

Al-Shimary, A. O. (2000). Extraction and purification of lignan compound from flax seed (*Linum usitatissimum*) and study its anti-mutagenicity on different systems. M.Sc. thesis, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Altwegg, M.; Murray, P.; Baron, R.; Pfaller, M.; Ternover, F. and Yolken, R. (1999). Manual of Clinical Microbiology. 7th ed., Americ. Soc. for Microb., Washington. PP. 507-517.

Ang-Lee, M. K.; Moss, J. and Yuan, G. S. (2008). Herbal medicines and perioperative care. JAMA, 28: 208-216.

Aroo, R. J.; John, J. M.; Eduard, A. H.; De Woard, M.; Els Van, W.; Peter, M.; Van, G.; Swolfs, E. M.; Klunder, J. H.; Anton, F. C. and George, J.W. (1995). Methanol

extract of *Tagetes patula* plant was assayed against phytopathogenic fungi. Photochemistry, 38 (5): 1193-1197.

Assumta, H. (2006). Marigold: A little ray of sunshine. Aust. Cent. for Compl. Med., 25(3): 214.

Azhar, L. A. (1998). The effect of some Iraqi plants on some pathogenic bacteria. M.Sc.Thesis, Dept. of Microbiology, College of Science, University of Babylon, Iraq.

Ballesta, M. C.; Perez, L.; Hernandez, M.; Berenguer, C.; Garcia, N. and Carvajal, M. (2008). Agricultural practices for enhanced human health. Photochemistry Rev., 7 (2): 251-260.

Bawle, E. V.; Weiss, L. and Conard, J. V. (1998). Adult and two children with fetal methotrexate syndrome. Teratology, 57 (2): 51-55.

Beneke, E. S. and Rogers, A. L. (1980). Medical Mycology manual, with human mycosis monograph. 4th ed. Minneapolis Burgess Publishing Comp., P: 20-75.

Bjornsdottir, S.; Gottfredsson, M.; Thorisdottir, A. S.; Gunnarsson, G. B. and Kristjansson, M. (2005). Risk factors for acute cellulites of the lower limb: a prospective case-control study. Clin. Infect. Dis., 41(10): 1416-1422.

Bodey, G. P.; Boliver, R.; Fainstein, V. and Jadeja, L. (2007). Infections caused by *Psedumonas aeraginosa.* Rev. Infect. Dis., 5 (2): 276- 313.

Bond, K. and Goldblat, L. (2007). The Pharmacology of Chinese Herbs. 2nd ed., CRC Press, Boca Raton, pp. 457–483.

Bown, D. (1995). The encyclopaedia of Herbs and Their Use, Dorling Kindersley, London, pp.44-48.

Carl, W. and Emrich, L. S. (1991). Management of oral mucositis during local radiation and systemic chemotherapy: A study of 98 patients. J. Prosthet. Dent., 66: 361–369.

Cappauecino, J. and Shermany, N. (1987). Microbiology, a Laboratory Manual. 2nd ed., Benjamin, Cummings Publishing, Inc. California, pp.53-171.

Chevallier, A. (1996). The Encyclopedia of Medicinal Plants. Dorling Kindersley Limited, London, pp.113-120.

Chinery, R. (1997). Antioxidants enhance the cytotoxicity of chemotherapeutic agents in colorectal cancer. Nat. Med., 3(11): 1233-1241.

Cowan, M. (1999). Plant products as antimicrobial agents. Americ. Soc. for Microbiol., 12: 564-582.

Daniel, L.; Stuberg, M. D.; Mare, A.; Penrod, M. D.; Richard, A. and Blantny, M. D. (2002). Common bacterial skin infections. Americ. Family Physicians, 66: 119-124.

Dave, B. (2003). Isolation, characterization and assessment of secondary metabolite from plants for use in human health. National Bioproducts and Bioprocess Program. Canada.

Dewick, P. (1998). Medicinal natural products. A biosynthetic approach. Department of pharmaceutical sciences, University of Nottingham, UK.

Dharmagadda, V.; Naik, S.; Mittal, P. and Vasudevan, P. (2005). Larvicidal activity of *Tagetes patula* essential oil against three mosquito species. Bioresource Technol., 96: 1235-1240.

Digrki, M.; Alma, M.; Ilcim, A. and Sen, S. (1999). Antibacterial and antifungal effects of various commercial plants extract. Pharm. Biol., 37: 216-220.

Dimo, T.; Mguelefack, T. B. and Tan, P. V. (2003). Possible mechanism action of flavonone extract from marigold. Phytother. Res., 17: 1135-1139.

Djehuty, M. R. (2007). *Candida albicans*: Yeast infection. Comp. of Derm. Herbs, 17: 114-120.

Donald, R. B. and Cristobal, M. (2000). Antioxidant activities of flavonoids. Department of environmental and molecular toxicology, Oregon State University.

Duthie, G. G. and Crozier, A. (2000). Plant derived phenolics antioxidants. Cur. Opin. in Lipiaol., 11: 43-47.

Duran, V.; Matic, M. and Jovanovc, M. (2005). Results of the clinical examination of an ointment with marigold extract in treatment of venous leg ulcers. Int. J. Tissue React., 27 (3): 101-106.

Dutta, B. K.; Karmakar, S.; Naglot, C.; Aich, A. J. and Begam, M. (2007). Anticandidial activity of some essential oils of a mega biodiversity hotspot in India. Mycoses, 50 (2): 121-124.

Eckardt, F. and Haynes, R. H. (1981). Quantitation measures of induced mutagenesis in short term tests for chemical carcinogens. 2nd ed., Springer-verlag, NewYork, Berlin.

Edward, F. and Gilman, K. (1999). *Tagetes patula* L. (French marigold). Inst. of Food and Agri. Sci., 569: 1-3.

El-Astal, Z. Y.; Ashour, A. E. and Kerrit, A. M. (2005). Antimicrobial activity of some medicinal plant extracts in Palestine. Pak. J. Med. Sci., 21(2): 187-193.

Essawi, T. and Srour, M. (2000). Botanical medicine for thyroid regulation. J. Altern. and Comp. Med., 3 (12): 107-112.

Finegold, S. M.; Nartin, W. J. and Scott, E. G. (1982). Antimicrobial susceptibility tests and assays. In; Baily and Scott's Diagnostic microbiology. 6th ed., Chap., (36) P: 385-404.

Francisco, G. (2008). Four hundred flowers: The Aztec Herbal Pharmacopoeia, 1st ed. Mexico.

Faizi, S.; Siddiqi, H.; Bano, S.; Naz, A.; Lubnaa, K. M.; Nasim, S.; Riaz, T.; Kamal, S.; Ahmad, A. and Khan, S. A. (2008). Antibactrerial and antifungal activities of different parts of *Tagetes patula*: Preparation of patuletin derivatives, Pharm. Biol., 46(5): 309-320.

Frazier, W. C. and Westhoff, D. C. (1987). Food Microbiology. 3rd ed. Mc Graw. Hill Book Co. New York.

Gann, P. H.; Staba, E. and Giovansucci, E. (1999). Lower prostate risk in men with elevated plasmalycopene levels. Pros. Anal. Can. Res., 59: 1225-1230.

Garry, G. D.; Susan, J. D. and Janet, A. M. (2000). Plant polyphenols in cancer and heart diseases: Implications as nutritional antioxidants. Nut. Res., 13: 79-106.

Ghoshal, S. B. and Lakshm, L. (1996). Antibiotic activity of *piper longum* fruits against entombed histolytic *in vitro* and *in vivo*. J. ethnopharmacol., 50: 167-170.

Grieve, M. (2007). Marigold herb profile and information. A modern Herbal. England, pp.1-13.

Guinot, P.; Gargadennec, A.; Valette, G.; Fruchier, A. and Andary, C. (2007). Primary flavonoids in marigold dye: Extraction, Structure, and Involvement in dyeing process. Phytochem. Anal., 19: 46-51.

Gupta, Y. C.; Sharma, Y. D. and Pathania, N. S. (2007). Tagetes species. Wikipedia, 21: 9.

Hadjiakhoondi, A.; Vatandoost, H.; Knanavi, M.; Abaee, M. R. and Karami, M. (2005). Biochemical investigation of different extracts and activity of *Tagetes minuta* L. Iraian J. of Pharm. Sci., 1(2): 81-84.

Hansel, R. and Stichergger, E. (1999). Pharmakognosie-hytopharmazie, 6th ed., Springer-Verlag, Berlin. pp. 692-695.

Harborne, J. B. (1984). Phytochemical Methods, (A guide to Modern Technique of Plant Analysis), Chapman Hall, London.

Hartmann, T. (1996). Diversity and variability of plant secondary metabolism: a mechanistic view. Entomol. Exp. Appl., 80:177-188.

Hay, R. J. (1993). Histoplasmosis. Semin Dermatology, 12: 310-314.

Heinrich, U. (2003). Supplementution with beta-carotene or a similar amount of mixed carotenoids protects humans from UV-induced erytheme. J. Nutr., 133: 98-101.

Hemphill, L. and Cobiac, L. (2006). Health benefits of herbs and spices: the past, the present, the future. The Medical. J. of Austr., 185: 5-6.

Hirano, T.; Gotoh, M. and Oka, K. (1994). Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. Life Sci., 55: 1061-1069.

Hollman, P. C. and Katan, M. B. (1999). Dietary flavonoids: intake, health effect and bioavailability. Food and Chem. Toxicol., 37: 937-942.

Hudson, L. and Hay, F. C. (1980). Practical Immunology. 2nd ed., Black Well Scientific Publ, London.

Humphery, H.; Green, W. D.; Reutherer, J., and Slack, R. (1997). Medical Microbiology. 15th ed., Churchill Living Stone, New York, USA.

Ibrahim, N. A. (2003). *In vivo* and *in vitro* immunological and cytogenetic studies of *calendula officinalis* effects on albino male mice and acute myeloid leukemic cells. M.Sc. Thesis, College of Science, University of Al-Nahrain, Iraq.

Iqbal, M. P. (2003). Mechanism of drug resistance in cancer cells. J. Med. Sci., 19 (2): 118-127.

Ivancheva, S. and Zdravkova, M. (1993). Flavonoids in *Tagetes patula*. Fitotherapia, 64: 555.

Janssen, A. M.; Scheffer, J. C. and Svendesn, A. S. (1987). Antimicrobial activity of essential oils. Plant Medical, 53: 395-398.

Jawad, A. M.; Jaffer, H. J.; Al-naib, A. and Naji, A. (1988). Antimicrobial activity of sesquiterpene lactone and alkaloid fractions from Iraqi-plants. Int. J. Crude Drug. Res., 26 (4): 185-188.

Jawetz, M. D.; Melinich, J. L. and Adelberg, E. A. (1998). Medical Microbilogy. 21st ed., Prentice-Hall, USA, pp. 2587-2591.

Jay, W. and Ogbru, O. (2008). Methotrexate. Med. Pharm., 26: 154-163.

Jian, Z.; Wei, L.; Goo, Y. and Wang, Q. (2007). Phenolic, flavonoid, and lutein ester content and antioxidant activity of 11 cultivars of Chinese marigold. Americ. Chem. Soc., 55 (21): 8478- 8484.

John, W. (1996). Principles and Practice of Clinical Mycology. Edited by C. C. Kibbler; D. W. R. Mackenzie and F. C. Odds.

Johnson, E. J.; Hammond, B. R.; Yeum, K. J.; Qin, J.; Dong Wang, X.; Castaneda, C.; Snodderly, D. M. and Russell, R. M. (2000). Relation among serum and tissue

concentrations of lutein and zeaxanthin and macular pigment density. Americ. J. Chin. Nutre., 71: 1555-1562.

Jolivet, J.; Coam, K. H.; Curt, G. A.; Clendeninn, N. J. and Chabner, B. A. (1995). The pharmacology and clinical use of methotrexate. New Eng. Med., 309: 1192-1194.

Kagan, J. (1991). In progress in the chemistry of natural products naturally occurring di and trithiophenes. Springer. Verlag. Wien. New York, 56: 87-169.

Kasahara, Y.; Yasukawa, K.; Kitanaka, S.; Khan, T. M. and Evans, F. J. (2002). Effect of methanol extract from flower petals of *Tagetes patula* L. on acute and chronic inflammation model, Phytother. Res., 16: 217-222.

Kasumov, M. N. (1991). New sources of plant raw material for production of yellow food dye. Dokl. Akade. Neuk. Azerb., 9: 60-64.

Kaushik, P. and Dhiman, A. K. (2000). Medicinal Plants and Raw Drugs of India. Bishen Singh, Mahendra Pal Singh. Dehradun: New Cannaught Place.

Khachik, F.; Steck, A. and Pfander, H. (1999). Isolation and structural elucidation of lutein from Marigold flowers, kale, and human plasma. J. Agric. Food Chem., 47: 455-481.

Khan, M. T.; Potter, M. and Birch, I. (1996). Podiatric treatment of hyperkeratotic plantar lesion with marigold, Phyother. Res., 10: 211-214.

Kirtikar, K. R. and Basu, B. D. (2000). Indian medicinal plants. Prok. Allahbad. India, 3: 1951-1952.

Klareskog, L.; Heijde, D.; Jager, J. P.; Gough, A.; Kalden, J.; Malaise, M.; Martin, M. E.; Pavelka, K.; Sany, J.; Settas L; Wajdula, J.; Pedersen, R.; Fatenejad, S. and Sanda, M. (2004). Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. Lancet, 363 (9410): 675- 681.

Kovacs, S. O. and Hruza, L. L. (1995). Superficial fungal infections. Getting rid of lesions that don't go away. Postgrad. Med., 98(6): 61-75.

Krinsky, N. I. (2003). Biological mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu. Rev. Nature., 23: 171-201.

Kundig, C. H.; Haimeur, A.; Legare, D.; Papadopoulou, B. and Ouellette, M. (1998). Increased transport of pteridines compensates for mutations in the high affinity folate transprter and contributes metjotrexate resistance in the protozoan parasite *Leishmania tarentolae*. Embo. J., 18: 2342-2351.

Madamombe, I. and Afolayan, A. (2003). Evaluation of antimicrobial ctivity of extracts from South African. Pharmaceut. Biol., 41: 199-202.

Margl, L.; Tei, A.; Gyurjan, I. and Wink, M. (2002). GLC and GLC-MS analysis of thiophene derivatives in plant and in *in vitro* culture of *Tagetes patula* L.(Asteraceae), Z. Naturforsch., 57: 63-71.

Maria, R.; Velasco, J.; Hernandez, J.; Pecheneda, M.; Janne, R.; Carmona, J.; Diaz, T. and Morales, A. (2006). Chemical composition and antibacterial activity of the essential oil of *Tagetes patula* L. (Asteraceae) collected from the Venezuela. Res. Latinoamer. Quim., 34 (3): 32-36.

Marris, F. (2008). *Tagetes patula* L. (French marigold). Plants for healthcare professionals. London: Churchill Livingstone, pp: 7-8.

Martin, K. W. and Ernst, E. (2003). Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials, J. Antimicrob. Chemo., 51(2): 241-246.

Mattes, R. D. (1994). Prevention of food aversions in cancer patients during treatment. Nutr. Cancer, 21: 13–24.

Mejumdor, S. and Aggarwal, B. B. (2001). Methotrexate suppresses NF-KB activation through inhibition of IKBX phosphorylation and degradation. J. of Immunol., 167: 2911-2920.

Melissa, H. (2007). Marigold: History and culture calendula and tagetes varieties of marigolds are useful. Int. J. Toxicol., 20 (2): 13-20.

Meyer, K.; Schwartz, J.; Crater, D. and Keyes, B. (1995). *Zingiber officinale* (ginger) used to prevent 8-Mop associated nausea. Dermatol. Nurs., 7: 242–244.

Mills, S. Y. (2000). Medicinal Herb Garden, 3rd ed., Cambridge University Press, pp. 84- 92.

Mitscher, L. A.; Leu, R.; Bathala, M. S.; Wu Wu, W. N.; Beal, J. L. and White, R. (1972). Antimicrobial agents from higher plants, Fitoteropia, 35(2): 157-166.

Mohanasun, M. D.; Michael, C. T. and Bear, D. (2007). Marigold (*Calendula officinalis* L.), Herb Med., 14: 1-15.

Mothana, R. A. and Lindequist, U. (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. J. Ethnopharmacol., 96(1-2): 177–181.

Muller, G.; Kirk, R. and Scott, D. (1989). *Small Animal Dermatology*. 4th ed. Philadelphia: WB Saunders. Nail (claw) Diseases; p 820-825.

Murakami, A.; Ohigashi, H. and Koshimizu, K. (1994). Possible antitumor promoting properties of their active conational of that food items and some of their active constituents. Pac. J. Clin. Nut., 3: 185-191.

NCCLS. (2005). National Committee for Clinical Laboratory Standards: Performance Standards for Antimicrobial Susceptibility Testing; MIC testing. Doc. M., 25: 87-162.

Nishifuji, K.; Sugai, M. and Amagai, M. (2008). Staphylococcal exfoliative toxins: "Molecular scissors" of bacteria that attack the cutaneneous defence barrier in mammals. J. of Dermatol. Sci., 49(1): 21-31.

Okemo, P.; Mwatha, W.; Chhabra, S. and Fabry, W. (2001). The kill kinetics of *Azadirachta andica* (meliaceae) extracts on *Staphylococcus aureus, E. coli, Pseudomonas aeriginosa* and *Candida albicans*. Afr. J. Sci. Tech., 2: 113-118.

Pathak, A.; Revatkar, S. and Chande, C. (1999). Prevalence of biotype and serotypee of *Candida albicans* among clinical isolates. Indian J. Med., 109: 46-48.

Pauline, G.; Gargadennec, A.; Valette, G.; Fruchier, A. and Andary, C. (2008). Primary flavonoids in marigold dye: extraction, structure, and involvement in the dyeing process. Phytochem. Anal., 19: 46-51.

Peter, W. and Pearson, A. E. (1971). The Laboratory animal's principles and practice. Academic Press. New York Pp: 226-234.

Piccaglia, R.; Marotti, M. and Grandi, S. (1998). Lutein and lutein ester content in different types of *tagetes patula* and *tagetes erecta*, Ind. Crops Prod., 8: 45-51.

Ploeg, N. and Antoon, T. (2000). Effects of amending soil with *tagetes patula* (singl gold) on *Meloidogyne incognita* infestation of tomato, Nematol. B. Res., 2(5): 489-493.

Podschun, R. and Ullmann, U. (1998). *Klebsiella spp*. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin. Microbiol. Rev., 11 (4): 589-603.

Prabuseenivasan, S.; Jayakumar M. and Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. BMC comple. and alternat. Medici**.**, (6): 39-42.

Rai, M. K.; Kaushal, K. S. and Acharya, D. (2002). *In vitro* effect of five asteraceous essential oil against *Saprolognia ferax*. The antiseptic, 99 (4): 136-139.

Rauha, J. P.; Remes, S.; Heinonen, M.; Hopia, A.; Kahkonen, M. and Kujala, T. (2000). Antimicrobial effects of Finnifh extract containing flavonoids and other phenolic compounds. Int. J. F. Microbiol., 56(1): 3-12.

Reverned, E. A. (1998). Herbal Combinations, in the Herbal Encyclopedia, London Press, U.K.

Richard, C. (2004). Lutein from *Tagetes erecta*. Chem. and Tech. Asse., 63 (5): 1-5.

Roberts, S. and Chambers, S. (2005). Diagnosis and management of *Staphylococcus aureus* infections of the skin and soft tissue. Intern. Med. J., 2: 97-105.

Robin, D.; Kathleen, R. and Shoor, M. (2006). Methotrexate for rheumatoid arthritis. Med. Rheuma., (23): 52-62.

Rose, M.; Perez, G.; Heliodoro, H. and Sergio, H.G. (2006). Antioxidant activity of *Tagetes erecta* essential oil. J. Chil. Chem.. Soc., 51 (2): 883-886.

Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology, 4th ed., Mc Crow Hill, pp. 22-25.

Sagar, D.; Naik, S. N.; Rout, P. K. and Rao, Y. U. (2005). Composition of essential oils of *Tagetes patula* L. growing in Northern India. J. Ess. Oil Res., 17: 446-448.

Saleem, R.; Ahmad, M.; Naz, A.; Siddiqui, H.; Ahmad, S. I. and Faizi, S. (2004). Hypotensive and toxicological study of citric acid and other constituents from *Tagetes patula* roots. Arch. Pharm. Res., 27(10): 1037-1042.

Scalbert, A. (1991). Antimicrobial properties of tannins. Phytochemistry, 30: 3875-3883.

Schaller, M.; Jaunschke, E.; Schackert, C. and Korting, H. C. (2001). Different isoforms of secreted as partly proteinases are expressed by *Candida albicans* during oral cutaneous candidosis *in vivo*. J. Med. Microbiol., 50:743-747.

Scheper, J. (2003). Tagetes *spp.* Florida Data, Talla Hassee, USA, pp. 1-5.

Schneider, D. J.; Tracy, P. B.; Mann, K. G. and Sobel, B. E. (1998). Differential effects of anticoagulants on the activation of platelets. Chem. Abstr. 128: 97525.

Serra, M.; Reverter, B. G.; Maurici, D.; Benini, S.; Shen, J. N; Claro, T.; Hathinger, G. M.; Manzro, L.; Pasello, M.; Scotlandi, K. and Picci, B. (2004). Analysis of dihydrofolate and reduced folate carrier gene status in relation to methotrexate resistance in osteosarcon and cells. Ann. of Oncol., 15: 151-160.

Shapoval, E. S.; Silveria, S. M.; Miranda, M. L.; Alice, C. B. and Henniques, T. (1996). Evaluation of some pharmacological activities of *Eugenia uniflora*. J. Ethnopharmacol. 44:124-136.

Shihata, I. M. (1951)**.** A pharmacological study of *Anagalis arvensis*, M. D. Vet. Ph.D.Thesis, Cairo University, Egypt.

Singh, R.; Jain, A.; Panwar, S.; Gupta, D. and Khare, S. K. (2005). Antimicrobial activity of some natural dyes. Dyes Pigm., 66: 9-102.

Smolenski, S. J.; Silinis, H. and Farans-Worth, N. R. (1972). Alkaloids screening, lidiaa, 35: 31-34.

Stahl, E. (1969). Thin-Layer-Chromatography, A laboratory Hand Book. 2th.ed. Translated by Ashworth, M. R. F. Spring. Verag, Berlin, Heidelberg, New York, U.S.A.

Stoppler, M. C.; William, C. and Shiel, J. (2007). Staph infection (*Staphylococcus aureus*) symptoms, causes, and treatment. Med. Net., 5: 21-25.

Szarka, S. Z.; Hethelyi, E. B.; Lemberkovics, E.; Kuzvkina, I. N.; Balvanyos, I. and Szoke, E. (2007). Investigation of volatile compounds in tagetes species. Russian Acad. of Sci., Moscow, 35: 34-37.

Taiz, L. and Zeiger, E. (2002). Plant Physiology. Sinauer Associates, Inc. Publishers. Sunderland.

Tapsell, L. G. (2006). Health benefits of herbs and spices: the past, the present, the future, Med. J. Aust., 8: 463-473.

Tayler, R. S. L.; Edel, F.; Manandhar, N. P. and Towers, G. N. (1996). Antimicrobial activity of Southern Nepal medicinal plants. J. Ethnopharmaco., 50: 97-102.

Threlkeld, D. S. (2000). Antineoplastics, Antimetabolites, Methotrexate. In Facts and Comp. Drug Infor. St., 35: 653–654.

Todar, K. (2004). *Psedumonas aeraginosa*. University of Wisconsin, Med. Dept. of Bacteriol., pp.1-15

Tomesi, C. N. ; Viol, A. A.; Buschi, C. A. , Rofi, R. D. ; Schteingart, G. A. and Pomilio, A. B. (1996). Antimicrobial screening of some Argentine higher plants. Part Π. Fitoteropia, 57: 46-50.

Trease, J. E. and Evans, W. C. (1987). Pharmacognocy. 13th ed. Balliere. Tindall, pp. 62-68.

Tsuda, H.; Ohshima, Y.; Nomoto, H.; Fujta, K.; Matsudo, E.; Jigo, M.; Takasuka, N. and Moore, M. A. (2004). Cancer prevention by natural compounds. Durg Metab. Pharma., 19 (4): 245-263.

USDA. (2008). Taxon: *Tagetes patula* L., http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.com.

Vasilenko, Y. K.; Bogdanov, A. N.; Frolova, L. M. and Frolova, A. V. (1990). Hepatoprotective properties of preparations from spreading marigold, Khim. from Zh., 24 (1): 53-56.

Vasudevan, P.; Kashyap, S. and Sharma S. (1997). Tagetes: A multipurpose plant. Bio. Technol., 62: 29-35.

Vodopich, D. and Moore, K. (1992). Biology. Laboratory Manual 3rd ed. pp: 112-124.

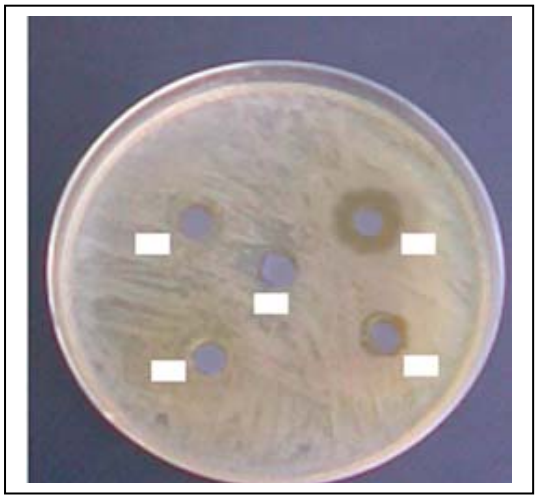
Voutilainen, S.; Nurmi, T.; Mursu, J. and Rissanen, T. H. (2006). Carotenoids and cardiovascular health. Americ. J. Clin. Nutr., 83 (6): 1265-1271.

Wenying, R.; Zhenhua, Q.; Hongwei, W.; Lei, Z. and Zhang, O. (2003). Flavonoids: promising anticancer agents. Med. Res., 23 (4): 519- 534.

Yadav, I. S.; Thakur, S. and chadha, P. (2003). *Chyawanprash awaleha*: A genoprotective agent for Bidi Smokers. Int. J. Hum. Genet., 3: 33-38.

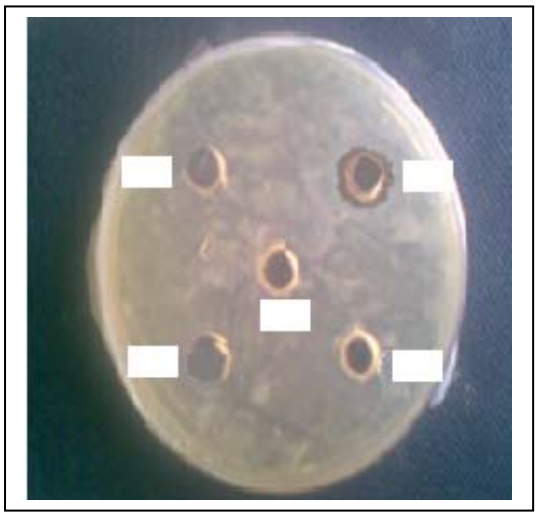
Yong-Suk, K. and Dong-Hwa, S. (2005). Volatile components and antibacterial effects of pine needle (*Pinus densiflora* S. and Z.) extracts. Food Microb., 22: 37-45.

Appendices

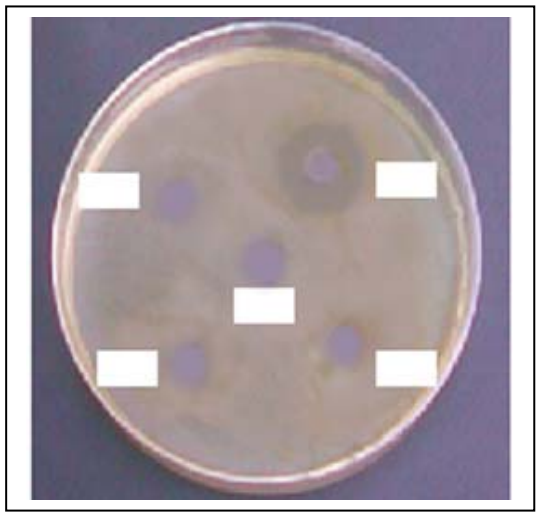


A

B

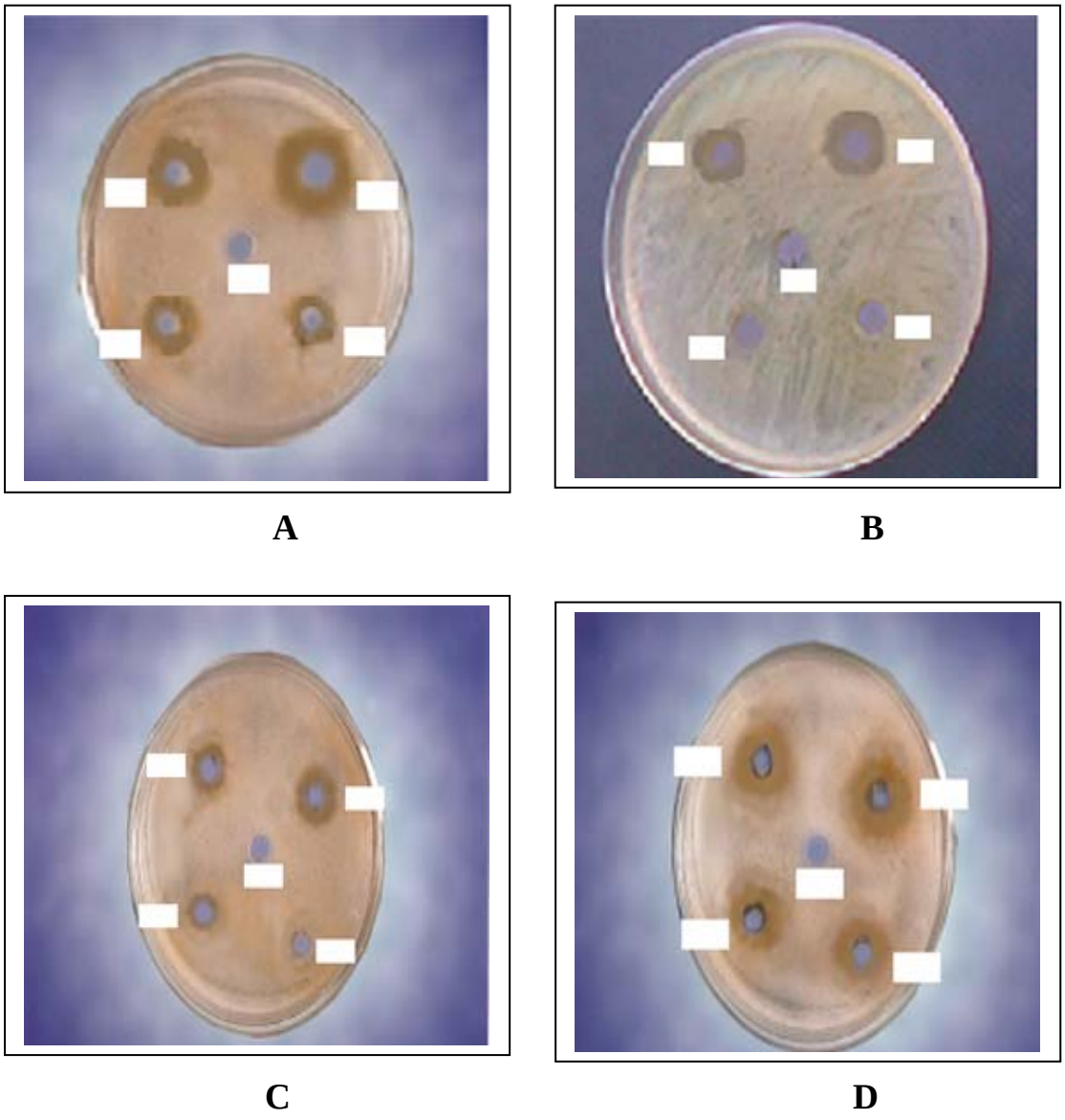


C



D

Appendix 1: Inhibition zones caused by applying essential oil extract against *S. aureus* (A), *P. aeruginosa* (B), *K. pneumoniae* (C), and *C. albicans* (D). 1=control, 2= Crude, 3=750 ppm, 4=500 ppm, and 5=250 ppm.



A **B**

C **D**

Appendix 2: Inhibition zones caused by applying methanol flower extract against *S. aureus* (A), *P. aeruginosa* (B), *K. pneumoniae* (C), and *C. albicans* (D). 1=control, 2=75 mg/ml, 3=50 mg/ml, 4=25 mg/ml, and 5=12.5 mg/ml.

Appendix 3: Survival index before and after incubation with *Bacillus spp.* [G3] cultures using different treatments

Time	Treatments						Mean
	1	2	3	4	5	6	
Before incubation	1.09 ±0.05	0.07 ±0.00	1.02 ±0.04	0.66 ±0.03	1.00 ±0.11	1.13 ±0.20	0.82 ±0.04
After incubation	1.16 ±0.06	0.14 ±0.02	0.96 ±0.03	0.56 ±0.02	1.00 ±0.11	0.96 ±0.12	0.79 ±0.03
Mean	1.12 ±0.05	0.105 ±0.02	0.99 ±0.02	0.61 ±0.03	1.00 ±0.11	1.04 ±0.05	
LSD value (0.05)	Treat:0.0129* Time:0.0074* Treat× Time:0.0366*						

* = (P≤0.05), 1 = Negative control, 2 = Positive control with MTX only, 3 = Treatment with flower extract, 4 = Treatment with flower extract before treatment with MTX, 5 = Treatment with a combination of flower extract and MTX, and 6 = Treatment with flower extract after MTX treatment.

Appendix 4: Survival index before and after incubation with *Arthrobacter spp.* [G12] cultures using different treatments.

Time	Treatments						Mean
	1	2	3	4	5	6	
Before incubation	1.22 ±0.07	0.18 ±0.01	1.19 ±0.04	0.92 ±0.02	0.96 ±0.02	1.07 ±0.05	0.92 ±0.04
After incubation	1.34 ±0.09	0.37 ±0.06	1.03 ±0.03	0.80 ±0.04	0.96 ±0.02	1.00 ±0.04	0.91 ±0.04
Mean	1.38 ±0.06	0.27 ±0.03	1.11 ±0.03	0.86 ±0.03	0.96 ±0.02	1.03 ±0.05	
LSD value (0.05)	Treat:0.0352* Time:0.0204^{ns} Treat× Time:0.065*						

* = (P≤0.05), ns= non-significant, 1 = Negative control, 2 = Positive control with MTX only, 3 = Treatment with flower extract, 4 = Treatment with flower extract before treatment with MTX, 5 = Treatment with a combination of flower extract and MTX, and 6 = Treatment with flower extract after MTX treatment.

Appendix 5: Survival index before and after incubation with *Brevibactrium spp.* [G27] cultures using different treatments

Time	Treatments						Mean
	1	2	3	4	5	6	
Before incubation	1.16 ±0.06	0.14 ±0.01	1.04 ±0.11	0.94 ±0.02	1.00 ±0.11	1.09 ±0.04	0.89 ±0.08
After incubation	1.22 ±0.08	0.20 ±0.03	1.00 ±0.11	0.84 ±0.03	1.00 ±0.11	0.98 ±0.03	0.87 ±0.06
Mean	1.19 ±0.09	0.17 ±0.03	1.02 ±0.11	0.89 ±0.02	1.00 ±0.11	1.03 ±0.04	
LSD value (0.05)	Treat:0.022* Time:0.0127* Treat× Time:0.032*						

* = (P≤0.05), 1 = Negative control, 2 = Positive control with MTX only, 3 = Treatment with flower extract, 4 = Treatment with flower extract before treatment with MTX, 5 = Treatment with a combination of flower extract and MTX, and 6 = Treatment with flower extract after MTX treatment.

الـخلاصـة

في محاولة لمعالجة اصابة جلد الفئران وتقيم التاثير للنمو المكروبي لنظام بكتيري G-system باستخدام الزيت الاساسي والمستخلص الكحولي لازهار نبات *Tagetes patula* (القديفة او الجعفري) تم تنفيذ عدد من التجارب لهذا الغرض.

اشارت النتائج الى ان المستخلصين الزيتي والكحولي لازهار القديفة لهما فعاليه مضاده للبكتريا و الخميرة *Candida albicans*. تم الكشف الكيميائي لمكونات المستخلص الكحولي لازهار القديفة وقد احتوى على الفينولات والفلافينويدات و القلويدات و الصابونيات والتربينات.

اوضحت النتائج بان المستخلص الكحولي للازهار قد اعطى فعالية تثبيطيه اعلى مـن الزيتـي فـي نمـو *Staphylococcus aureus* و*Klebseilla pneumoniae* و*Pseudomonas aeruginosa* و *Candida albicans* ولم يعطي المستخلصين اي فعالية تثبيطيه في نمو *Escherichia coli*.

لوحظ أن المستخلصين الزيتي(غير المخفف) والكحولي (50mg/ml) لازهار القديفه لهمـا اعلـى فعاليـة تثبيطيه في نمو عزلة *S. aureus* المسببة لالتهاب الجلد, لذالك استعمل هذان المستخلصان على الفئران مختبريا. اظهر المستخلصان الزيتي والكحولي لازهار القديفة بان لهما تاثيرات مضاده للبكتريا والالتهابات عند مقارنتهمـا بمرهم علاج امراض الجلد الساماسايللين في الفئران المصابة بهذه البكتريا.

أظهرت النتائج بان المستخلص الكحولي لازهار القديفة غني بالفلافون. كما لم يظهر التركيـز المسـتخدم للمستخلص اي تاثير خلوي سـمي للنظـام البكتيـري [*Bacillus spp.* (G3), *Arethrobacter spp.* (G12) *Brevibacterium spp.* (G27)]. كما أن معاملات التداخل (بعد ومع وقبل) لـدواء Methotrexate بتركيـز 50µg/ml والمستخلص الكحولي بتركيز50mg/ml استعمل لتقليل تاثيرMethotrexate على النظام البكتيري.

//الاهداء

الى الله جل جلاله وروح الممصطفى (صلى الله عليه وأله وصحبه أجمعين)

الى بحر العطاء وقدوة المربين والاباء ..... ابي

الى الشمعة التي تنور حياتي .... أمي

الى رفاق الدرب ..... إصدقائي

الى كل من علمني ولو حرفا

الى سندي في الحياة .... اخي محمد واخوتي هاله ورشا

اهدي لهم ثمرة جهدي المتواضع .... كبعض من الوفاء

فرح

بِسْم لله لرحمن لرحيم

وأنزلَ الله عَلَيْك أَلْكتاب وأَلْحكْمةَ وعلمك

مّالم تكُنْ تعْلَم وكانَ فَضْلُ اللهِ عَلَيْك عظيماً

صدق لله لعظيم

سورة النساء

أية 113

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الأحيائية

فعالية بعض نواتج الايض الثانويه المستخلصه من ازهار *Tagetes patula* L. ضد الاصابه المكروبية

رسالة

مقدمة إلى كلية العلوم / جامعة النهرين

كجزء من متطلبات نيل درجة ماجستير في علوم التقانة الإحيائية

من قبل

فرح داود سالم السعدي

بكالوريوس تقانة احيائية - جامعة النهرين 2006

بأشراف

د. كاظم محمد ابراهيم

ذوالقعده
1430 هـ

د. منيره جلوب أسماعيل

تشرين الثاني
2009 م