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In vivo and *In vitro* Antimicrobial Activity of Flower and Callus Extracts of *Matricaria chamomilla* L. in The Treatment of Experimentally - Induced Skin Infections in Mice

A thesis

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Summary

The current study was designed to examine the antimicrobial activity of the chamomile (*Matricaria chamomilla*) water or ethanol extracts for both flowers and callus cultures initiated on germinated seeds. An attempt was carried out to treat the induced infected mice with some microorganisms causing skin infection namely *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Results were compared with some drugs used for skin treatment namely, Cefotaxime sodium, Acetic acid, Fusidic acid and Clotrimazol. Callus was initiated and maintained on Murashige and skoog, 1962 medium (MS) supplemented with a combination of 1.0 mg/l Benzyl adenine (BA) and 0.1 mg/l 2,4-Dichlorophynoxyacetic acid (2,4-D). Results revealed that water or ethanol extracts have an inhibitory effects on the microorganisms under investigation. Extracts were containing azulene and apigenin compounds, after detection and quantitation using HPLC techniques. Ethanol flower extract was more effective against the studied microorganisms than water extracts. Meanwhile, callus ethanol extract was found more inhibitory to the microorganisms than water and ethanol flowers extracts. Thus, ethanol callus extract was applied at a concentration of 40 mg/ml for the treatment of induced skin infection of mice then compared with the skin drugs available in the pharmacies. A mixture of ethanol callus extract and Cefotaxime sodium at a percentage 50% was the most effective treatment against tested bacteria. Ethanol callus extract at a concentration of 40 mg/ml was effective in curing of mice skin infected with *C. albicans*.

In regards to the use of antimicrobial drugs in the experimentally – induced skin infection, further studies are required to test whether these sults represent a novel finding or an experimental error.

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List of Abbreviations

Abbreviation	Full name
2,4-D	2,4-dichlorophenoxyacetic acid
α	*Alpha*
BA	Benzyl adenine
°C	Degree Celsius
C. albicans	*Candida albicans*
CFU	Colony Forming Unit
D. W.	Distilled Water
DWT	Dry weight
G+ve	Gram positive
G–ve	Gram negative
HPLC	(High-Performance Liquid Chromatography)
hrs	Hours
LSD	Least Significant Differences
MS	Murashige and Skoog medium
M. chamomilla	*Matricaria chamomilla*
mAU	milli Amber Unit
n	Number of replicates
NaOCl	Sodium hypochlorite
P. aeruginosa	*Psedomonas aeruginosa*
PBS	Phosphate Buffer Saline

Abbreviation	Full name
PBPs	Penicillin-Binding Proteins
S. aureus	*Staphylococcus aureus*
S.D.	Standard deviation
SDA	Sabouraud Dextrose Agar
spp.	Species
SSSS	Staphylococcal Scalded Skin Syndrome

Chapter One

Introduction

and

Literature Review

1.1. Introduction

Chamomile includes Roman chamomile (*Anthemus nobilis*) which is a perennial, low-growing plant with a slightly stronger fragrance than the other type, German Chamomile (*Matricaria recutita*). Both have daisy-like flowers that bloom from late spring through late summer (Appelt, 1985).

The production of secondary metabolites *in vitro* is possible through plant tissue culture. *In vitro* study holds a potential for the production of high-quality plant based medicines. This can be achieved through different methods including micropropagation of cell lines which are capable of producing high yield of secondary compounds. The accumulation of secondary products in plant cell cultures depends on many factors including the composition of the culture medium and environmental conditions (Murch *et al*., 2000).

Extracts of Roman chamomile showed antitumor activity and extracts of German chamomile contain several antibacterial, antifungal and antiseptic properties. It is used against different types of bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae, Micrococcus spp.* and *Pseudomonas aeruginosa.* Both types of chamomile contain minute amounts of blue oil (azulene). This oil has neutralizing abilities on the toxins produced by various bacteria and therefore, assists in the healing process of wounds (Hewitt, 2001).

To evaluate the pharmatherapeutic quality, a sensitive, simple and accurate high-performance liquid chromatography (HPLC) method was developed for the quantitative analysis of the active compounds (Chun *et al*., 2006).

Matricaria recutita L. (*M. recutita*), also known as German chamomile, *Matricaria chamomilla*, *Chamomilla chamomilla*, *Matricaria*

suaveolens and chamomile, was used for centuries as a medicinal plant, mostly for gastrointestinal ailments and skin injuries and problems. In Germany it is used in wound care, where its effectiveness was reported. Aromatherapy with chamomile essential oil was found to be effective in healing episiotomy wounds (Shivananda *et al*., 2007). Therefore, this plant requires further investigations for its potential therapeutic uses.

The aims of this work were:

1- Comparison between the antimicrobial activity of flower and callus extracts.

2- Comparison between the activity of chamomile flower extracts and some drugs used for treatment of skin infection.

3- Administration of chamomile flower and callus extracts on infected mice skin in an attempt to treat the infection caused by the above mentioned pathogens.

1.2. Literature review

1.2.1. Herbal medicine

Medicinal plants are important in nature, the herbs chemical components are the most important for pharmaceutical companies. People are interested in medicines prepared from plants due to their little side effects, cheap and almost available compared with synthetic drugs. This may be because of the low concentrations of the active compounds found in plants which the human body would need (Mackin, 1993).

Chamomile, (*Chamomilla recutita*), the sun-loving plant of the plains, is rich in active ingredients and has remained one of the most popular herbs since ancient times. There are different classes of active constituents, which have been isolated and used individually in medical practice and cosmetics. The plant contains 0.24%–1.9% volatile oil, which is a wonderful blend of different individual oils. This oil, extracted from flower heads by steam distillation, can range in color from brilliant blue to deep green when fresh but fades over time to dark yellow. Despite fading, the oil does not lose its potency. The oil contains a-bisabolol (up to 50%) chamazulene cyclic sesquiterpenes, which directly reduce inflammation and are mild antibacterials. The essential oil also contains bisabolol oxides, farnesene and spiro-ether, which have anti-inflammatory and antispasmodic actions (Hoffmann, 1995).

Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998).

Herbalists tend to use extracts from parts of plants, such as the roots or leaves but not isolate particular phytochemicals (Andrew and Catherine, 1999).

The return to natural plant drugs has taken specific consideration and chamomile is widely used for its potential clinical and therapic benefits. Some of its clinical benefits are used as anxiolytic, spasmolytic, sedative, anti-allergic, anti-inflammatory, anti-ulcer, anti-bacterial, anti-fungal and anti-viral besides other properties (Gardiner, 1999; Nmecz, 2000).

The commercial development of plants as sources of antioxidants to enhance health and food preservation is of a current interest. Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases (Scalbert and Williamson, 2000).

Currently, about 25–30% of all drugs available as therapeutics are derived from natural products or natural product derivatives (Newman *et al*., 2003).

The World Health Organization (WHO) has shown that about 80% of the world's populations still rely on traditional medicine and natural products still play a very important role in the medicine (Clardy and Walsh, 2004).

Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives. In many cases, these substances (particularly alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many of the herbs and spices

used by humans to season food yield useful medicinal compounds (Lai, 2004; Tapsell, 2006).

Herbalism is a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. Herbalism is also known as botanical medicine, medical herbalism, herbal medicine, herbology and phytotherapy (Acharya and Shrivastava, 2008).

1.3. Plant description

Chamomile inflorescence (Fig. 1) is conical in shape and composed of two types of flowers; ray flowers (white color) and disc flowers (yellow color), it blossoms in early to mid-summer (Paulsen *et al*., 2001).

The plant grows vertically with light green stems, reaches up to three feet height. It prefers sandy soil and full sun. They grow in late spring or early summer. Flowers are harvested throughout summer when they are fully open (Joe and Teresa, 1999; Hewitt, 2001).

1.4. Distribution of chamomile

German chamomile is cultivated in Germany, Hungary, Russia, Argentina, Slovakia, Poland, Egypt and Iraq. German chamomile is native to Europe and western Asia and North America (Chakravarty, 1976; Salamon, 1992).

German Chamomile blossoms from May until August the disc flowers remain erect while ray flowers bow down. The flowers have a honey like smell. German Chamomile has smooth, green and thin stems and becomes (25 - 40) cm high. The leaves are gradually divided over the length of the stems. German Chamomile is an annual plant (Bremness, 2000).

It is found on roadsides, perennial forage crops, pastures, lawns, gardens, waste areas, and also along irrigation ditches, shorelines, streams and pond edges. In Alaska it appears to be restricted to areas with recent anthropogenic soil disturbance and little organic soil (Juras *et al.*, 2004; Parchoma, 2004).

-a- -b-

Fig. 1: Chamomile plant at full blooming stage (a) and dried flowers which used in this work (b).

1.5. Medicinal and aromatherapy uses

Chamomile is a multipurpose digestive aid to treat gastrointestinal disturbances including flatulence, indigestion, diarrhea, anorexia, motion sickness, nausea and vomiting. Chamomile is thought to heal ulcers and acts as an herbal bitter to stimulate the liver (Mann and Staba, 1986).

It is used to treat colic, croup and fevers. In women's health, it is used as an emmenagogue and a uterine tonic. Chamomile's essential oil is also a treatment for malaria and parasitic worm infections, cystitis, colds

and flu. The flowers are sometimes added to cosmetics as an anti-allergenic agent (Bown, 1995; Nemecz, 1998).

An infusion of the flowers is taken internally as an anodyne, anti-inflammatory, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, febrifuge, sedative, stomachic, tonic and vasodilator (Chiej, 1984; Duke and Ayensu, 1985; Bown, 1995).

Chamomile is used both internally and externally to treat an extensive list of conditions. It is used externally for wounds, ulcers, eczema, gout, skin irritations, neuralgia, sciatica, rheumatic pain, hemorrhoids, mastitis and leg ulcers (Berry, 1995).

Chamomile is also extensively consumed as a tea or tonic. It is used internally to treat anxiety, hysteria, nightmares, insomnia and other sleep problems, convulsions and even delirium tremens (Martens, 1995).

The flowers are also used externally to treat wounds, sunburn, burns, haemorrhoids, mastitis and leg ulcers. An infusion is particularly useful as a stomachic, nervine and sedative for young children, especially when they are teething (Bown, 1995).

Chamomile is used externally to treat diaper rash, cracked nipples, chicken pox, poison ivy, conjunctivitis and as a hair tint and conditioner. European oncologists use a chamomile mouthwash called Kamillosan to treat chemotherapy-induced mouth sores. The German has approved chamomile as an external use for inflammation of the skin, mucous membranes, bacterial skin diseases including those of the oral cavity and gums and respiratory tract inflammation. The German recommends chamomile to treat gastrointestinal spasms and inflammatory diseases of the gastrointestinal tract (Newall *et al.*, 1996).

German Chamomile has been known as an anti-inflammatory, antibacterial and bacteriostatic, wound-healing promoter and deodorant

has been used in combination with other herbal ingredients as mouthwash or dentifrice to reduce plaque growth and to improve the gingival health (Gultz *et al.*, 1998).

It is applied in domestic medicine in wetting cotton pads for topical application with the aim of helping the healing of skin wounds and cuts (Mills and Bone, 2000).

Chamomile has been used as a tea for various conditions and as a topical cream. Typical oral doses are (9 – 15) g/day. Gargles made from 8g chamomile flowers in 1,000 ml water have been used in trials (Mazokopakis *et al.*, 2005).

Poorly documented adverse reactions have been reported (eg, abortifacient effects, menstrual cycle irregularities, uterine stimulation with excessive use) (Newall *et al*., 1996; Wickline, 2004). As extracts of chamomile have demonstrated weak estrogenic activity (Rosenberg Zand *et al.,* 2001; Kassi *et al*., 2004). Use during pregnancy is best avoided.

1.6. Side effects and cautions

Allergic reactions to chamomile are commonly reported. Hypersensitivity reactions include anaphylaxis, dermatitis, lacrimation and sneezing. (Rowe, 1934; Benner and Lee, 1973; Jensen-Jarolim *et al.*, 1998).

Other anti-inflammatory effects are thought to occur via the influence of azulenes (chamazulene, prochamazulene, and guaiazulene) on the pituitary and adrenals, increasing cortisone release and reducing histamine release (Berry, 1995).

Manifestations of allergy are suggested to be dependent on the route of ingestion. Asthma, bowel cramps, diarrhea and vomiting related to oral intake via tea have been reported; inhalation of the essential oil predominantly manifests as asthma (Reider *et al*., 2000).

Chamomile contributed to the hemorrhaging is doubtful since the coumarin compounds in German chamomile lack the chemical configuration necessary for human anticoagulant activity (Majerus and Tollefsen, 2001).

Anaphylaxis resulting from chamomile-containing enemas has been documented (Maddocks-Jennings, 2004) as well as allergic conjunctivitis caused by chamomile-containing eye drops (Fraunfelder, 2004).

In another report, a 20-year-old woman with confirmed sensitivity to chamomile experienced acute rhinitis from merely using chamomile-scented toilet paper (Scala, 2006).

1.7. Active compounds of chamomile

Chamomile has exhibited both positive and negative bactericidal activity with *Mycobacterium tuberculosis, Salmonella typhimurium* and *Staphylococcus aureus.* About 120 chemical constituents have been identified in chamomile as secondary metabolites, including 28 terpenoids, 36 flavonoids and 52 additional compounds with potential pharmacological activity (Mann and Staba, 1986).

Components such as alpha-bisabolol and cyclic ethers are antimicrobial. Umbelfiferone is fungistatic while chamazulene and alpha-bisabolol are antiseptic (Duke and Ayensu, 1985). The chamomile extracts were found to have the most effective antileishmanial activity (Shnitzler *et al.,* 1996).

German chamomile flowers contain 0.24 - 2.0% volatile oil that is blue in color. The two key constituents, alpha-bisabolol and chamazulene, account for 50 - 65 % of total volatile oil content. Other components of

the oil include alpha-bisabolol oxide A and B, alpha-bisabolone oxide A, spiroethers (cis- and trans- en-yn-dicycloether), sesquiterpenes (anthecotulid), cadinene, farnesene, furfural, spathulenol and proazulene (matricarin and matricin). Chamazulene is formed from matricin during steam distillation of the oil. Yield varies depending on the origin and age of the flowers. European Pharmacopoeia recommends chamomile contains no less than 4 ml/kg of blue essential oil (European Pharmacopoeia, 1996).

Chamomile contains 0.5-3% flavonoids, the coumarins 0.1% derive from the cinnamic acid. It is worthy to emphasize the presence of dioxycoumarin, the essential oil content varies between 0.2 - 1.8% (Grieve, 1982).

Active substances in chamomile belong to chemically different structural types. The largest group of medically important compounds forming the essential oils includes chamazulene, apigenin, bisbololoxides and spathulenol. Flavonoids, coumarins and mucilages found in the chamomile flowers also have pharmacological effects (Maday, 1999).

Avallone *et al*., (2000) mentioned that the main active compounds in this essence are: Volatile oil (bisabolol, chamazulene), Sesquiterpene lactones, Hydroxycoumarins, Flavonoids and Mucilages.

Antispasmodic and anti-inflammatory activities have been attributed to some of chamomile's active ingredients, especially the flavonoids (anthemidin, apigenin, luteolin, among others), bitter glycosides, coumarins (herniarin and umbelliferone) and its volatile oils (containing alpha bisabolol and matricine, among others) (Ottariano, 1999; Presser, 2000; Brinker, 2001).

Azulene (Fig. 2) is an organic compound and an isomer of naphthalene, but whereas naphthalene is a colourless, azulene is dark blue. Its name is derived from the Spanish word azul, meaning "blue". The molecular formula $C_{10}H_8$, melting point 99-100 °C, boiling point 242 °C (Klaus and Klaus-Peter, 1990).

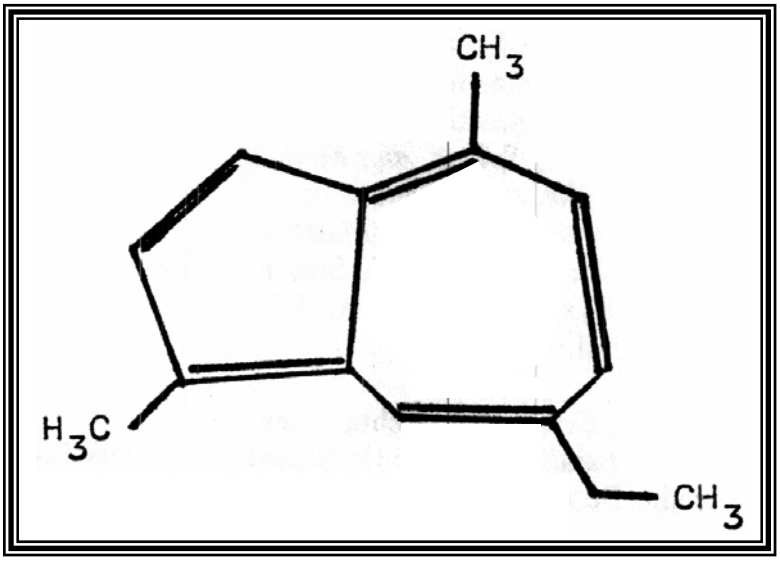

Fig. 2: Chemical structure of azulene (Klaus and Klaus-Peter, 1990).

Chamazulene, a product of the thermal decomposition of matricine, probably has the most anti-inflammatory activity. It has been shown to block the cyclo-oxygenase enzyme in the synthesis of prostaglandins. Its anti-inflammatory effects could be due to the inhibition of leukotriene formation (Dewick, 2002).

Chamazulene is present in German chamomile, but only traces occur in Roman chamomile (Dewick, 2002; Sandberg and Corrigan, 2002).

Apigenin (Fig. 3) according to Hertog *et al*., 1993 belongs to the flavone group of flavonoids. It is natural flavonoid present in the leaves

and stems of vascular plants, including fruits and vegetables (Lepley *et al.*, 1996).

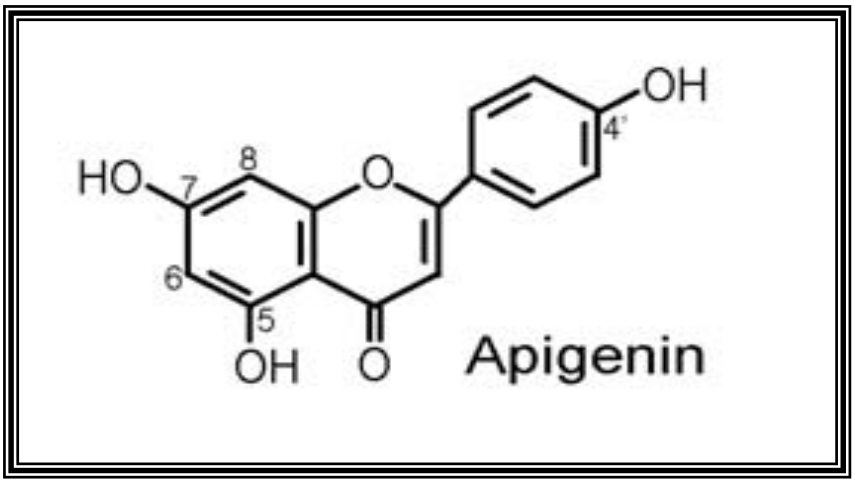

Fig. 3: Chemical structure of apigenin (Almela *et al.*, 2006).

Apigenin is found naturally in many fruits and vegetables, including apples and celery. It is found in several popular spices, including basil, oregano and parsley. The molecular formula $C_{15}H_{10}O_5$, Practically insoluble in water, moderately soluble in hot alcohol, soluble in dilute KOH (Budavari, 1997).

Apigenin is a major constituent of chamomile, which is recognized for its anti-inflammatory, antispasmodic and antibacterial effects. Chamomile preparations are also widely used in skin care products to reduce cutaneous inflammation and other dermatological diseases (Gruenwald *et al.*, 1998; Hoffman, 2000; Nemecz, 2000).

1.8. Mechanism of the active compounds of chamomile

Azulene may prevent histamine discharge from tissue by activating the pituitary-adrenal system, causing the release of cortisone (Stern and Milin, 1956). Azulene may prevent allergic seizures caused by histamine release, activating cellular resistance and speeding the process of healing (Meer and Meer, 1960). The azulene components of the volatile oil have anti-allergenic and anti-inflammatory actions (Farnsworth and Morgan, 1972).

The volatile oil components, chamazulene and α-bisabolol, have also demonstrated anti-inflammatory action by interfering with 5-lipoxygenase and cyclo-oxygenase production (Carle and Gomaa, 1992).

Apigenin may be an anti-inflammatory constituent, due to the water-soluble and lipophilic components. The flavones block arachidonic acid pathway by inhibiting phospholipase A, cyclo-oxygenase and lipoxygenase pathways (Hadley and Petry, 1999).

1.9. *In vitro* production of secondary metabolites

Plant cell cultures have proved to be an important tool for the study of secondary products biosynthesis. The secondary products may not be synthesized during certain lifetime of the plant. For example, flower pigments are only produced at a specific developmental stage, while a number of types of chemicals are rapidly synthesized. These differences within plants have often made the biosynthesis study difficult. Even for those secondary products that are synthesized more or less continuously, the rate of synthesis is frequently very low (Ramawat, 2008).

The production of the secondary metabolites *in vitro* is possible through plant tissue culture (Barnum, 2003; Karam *et al.*, 2003).

Theoretically by growing undifferentiated tissues *in vitro*, large amounts of biosynthetically active tissue could be generated. Thus, it would be possible to grow large quantities of biomass for the production of pharmaceuticals by fermentation using bioreactors (Stafford, 1991).

1.9.1. Callus cultures

Callus cultures are clumps of undifferentiated plant cells grown on nutrient media. The state of undifferentiated growth is maintained by the phytohormone balance, mainly auxins and cytokinins, added to the medium. In normal plant life, callus tissue is formed after wounding and this cell mass helps to close the wound rapidly. In an *in vitro* culture a tissue is wounded and the induced callus is further subcultured on nutrient media (Ramawat, 2008).

Sateesh (2003) divided callus growth to:

a. Lag phase, where cells prepare to divide.

b. Exponential phase, where the rate of cell division is increasing.

c. Linear phase, where cell division slows but the rate of cells expansion increases.

d. Deceleration phase, where the rate of cell division and elongation decreases.

e. Stationary phase, where the number and size of cells almost remain constant. Cells are harvested at this stage.

The optimum conditions for callus formation as well as the suitable sterilizing procedures and nutrient media, have to be determined empirically (Haq, 1993).

The productions of secondary compounds are more stable in callus cultures than in the suspensions. The callus stock provides the

material for the establishment of new suspensions. The degree of callus formation depends on the type of explants, plant species and plant growth regulators. Exogenous plant growth regulators (type, concentration, auxin to cytokinin ratio) are crucial for callus formation and this may depend upon the endogenous hormone content of the tissues under investigation (Pierik, 1987; Ramawat, 2008).

1.10. Increasing the yield of secondary metabolites

One of the major limitations of plant cell culture systems is the variable production of secondary products in many *in vitro* cultures of medicinal and aromatic plants. Variability is also evident at the biochemical level between cells in order to produce secondary metabolites. The low production may involve competition between primary and secondary pathways for key intermediates (Kurz *et al*., 1988).

Different types of cell lines may be isolated from variant cell suspension cultures using screening and selection techniques. The definition of screening here is the analysis of large number of cells or cell lines to identify rare individuals with high production ability of secondary metabolites. The selection is defined as the application of an environmental factor, which allows the survival or growth of cells with desired traits, such as, resistance to antibiotics, salinity, heavy metals or herbicides (Ramawat, 2008).

1.10.1. Addition of precursors

Addition of appropriate precursors to the culture media or related compounds, sometimes stimulate secondary metabolites production. This approach is advantageous if the precursors are inexpensive. For example,

phenylalanine is one of the biosynthetic precursors of rosmarinic acid (Zenk, 1977).

Addition of this amino acid to *Salvia officinalis* suspension cultures stimulated the production of rosmarinic acid (Tabata *et al*., 1971). Addition of phenylalanine to the callus cultures of *Taxus cupsidata* cells stimulated the biosynthesis of the anticancer compound and taxol (Fett *et al.*, 1995).

The exposure to physical abiotic elicitors such as UV light or chemical elicitors (NaCl) was may increase the yield of secondary metabolites. Biotic elicitors such as fungal toxins were added to some cultures and resulted in an increase of the production (Ramawat, 2008).

1.11. Skin infections

Skin infections are common and may be caused by bacteria, fungi or viruses. Breaks in the skin integrity, particularly those that inoculate pathogens into the dermis, frequently cause or exacerbate skin infections (Dagan and Bar-David, 1992).

Many types of microorganisms infect skin such as *S. aureus, Streptococcus pyogenes*, overgrowth of *Corynebacterium spp*., *P. aeruginosa, Mycobacterium spp., Clostridium perfringens, Klebsiella rhinoscleromatis* and others, in addition to fungi such as *C. albicans* and *Trichophyton rubrum* (Michael and Odell, 1998).

1.11.1. Bacterial skin infections

Bacterial skin infections include erythrasma and related diseases, impetigo, ecthyma, folliculitis, erysipelas and cellulitis. While there are certainly other bacterial skin infections, they are either uncommon or result from systemic illness (Trubo *et al.*, 1997).

1.11.1.1. *Staphylococcus aureus*

The bacterium *S. aureus* can infect other tissues when normal barriers have been breached (e.g., skin or mucosal lining). This leads to furuncles (boils) and carbuncles (a collection of furuncles). In infants *S. aureus* infection can cause a severe disease Staphylococcal Scalded Skin Syndrome (SSSS) (Curran and Al-Salihi, 1980).

The bacterium *S. aureus* is an aerobic and opportunistic pathogen, Gram positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. *S. aureus* is catalse positive and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish *Staphylococci* from *Enterococci* and *Streptococci*. A large percentage of *S. aureus* can be differentiated from most other *Staphylococci* by the coagulase test: *S. aureus* is primarily coagulase-positive (meaning that it can produce the enzyme "coagulase" that causes clot formation) while most other *Staphylococcus* species are coagulase-negative (Ryan and Ray, 2004).

1.11.1.2. *Pseudomonas aeruginosa*

The bacterium *P. aeruginosa* secretes a variety of pigments, including pyocyanin (blue-green), fluorescein (yellow-green and fluorescent, now also known as pyoverdin), and pyorubin (red-brown). King, Ward, and Raney developed Pseudomonas Agar P (aka King A media) for enhancing pyocyanin and pyorubin production and Pseudomonas Agar F (aka King B media) for enhancing fluorescein production (King *et al.*, 1954). *P. aeruginosa* is pathogenic only when introduced into areas devoid of normal defenses, like the mucous

membranes and skin when they are disrupted by direct tissue damage. The bacterium attaches to and colonizes the mucous membranes or skin, invades locally and produces systemic disease (Bodey, 1983; Jawetz *et al.*, 1995).

The bacterium *P. aeruginosa* is an opportunistic human pathogen, Gram negative, aerobic, rod-shaped bacterium with unipolar motility (Ryan and Ray, 2004). Although classified as an aerobic organism, *P. aeruginosa* is considered by many as a facultative anaerobe as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate as a terminal electron acceptor and in its absence it is also able to ferment arginine by substrate-level phosphorylation. Adaptation to microaerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*, like during lung infection in cystic fibrosis patients where thick layers of alginate surrounding bacterial mucoid cells can limit the diffusion of oxygen. (Collins, 1955; Hassett, 1996; Worlitzsch *et al.*, 2002; Cooper, 2003; Williams *et al.*, 2007).

1.11.2. Fungal and yeast infections

Fungi and yeast are capable of causing many different forms of skin infections, broadly referred to as dermatomycoses. *Candida albicans* and *Trichophyton*, *Epidermophyton*, *Microsporum* and *Malassezia* species are the most common infecting organisms. Many of the lesions caused by fungi are distinctive and diagnosis is primarily based on the presenting pattern rather than on culture or other test results (Kovacs and Hruza, 1995).

Candidiasis, commonly called yeast infection or thrush, is a fungal infection (mycosis) of any of the Candida species, of which *Candida albicans* is the most common (Walsh and Dixon, 1996).

1.11.2.1. *Candida albicans*

The fungus *C. albicans*, small amounts of which are normal in the mouth or digestive system. It only becomes a problem when it spreads out of control. An infection in the mouth or throat is termed: “thrush" (Bhutta, 1997). *C. albicans*, like many microorganisms, is a constituent of the normal body flora. While in the uterus, the body is generally free of germs; however during descent through the birth canal, or within hours after birth, the body becomes colonized by microbes. Both the skin and mucous membranes are colonized first because they are accessible to the external environment (El-Ebiary, 1997).

The fungus *C. albicans* appears as unicellular, yeast like fungi, reproduced by budding and it is polymorphic, able to change reversibly between round budding and elongated hyphen or filamentous growth. This morphological flexibility appears to be a key contributor to virulence (El-Barkani *et al*., 2000).

Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are however common in many human populations (Fidel, 2002; Pappas, 2006). While clearly attributable to the presence of the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes (Fidel, 2002).

1.12. Drugs used for skin infection

1.12.1. Cefotaxime sodium

To reduce the development of drug-resistant bacteria and maintain the effectiveness of Claforan (Cefotaxime sodium) and other antibacterial drugs, Claforan should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria. The bactericidal activity of Cefotaxime sodium results from inhibition of cell wall synthesis. Cefotaxime sodium has *in vitro* activity against a wide range of Gram-positive and Gram-negative organisms **(**Richmond and Sykes, 1973).

Claforan is indicated for the treatment of patients with serious infections caused by susceptible strains of the designated microorganisms in the diseases like in lower respiratory tract infections, genitourinary infections, gynecologic infections, bacteremia/septicemia, skin and skin structure infections, intra-abdominal infections, bone or joint and central nervous system infections **(**Gilbert *et al*., 2007).

1.12.2. Acetic acid 6%

Acetic acid, also known as ethanoic acid, is an organic chemical compound, giving vinegar its sour taste and pungent smell. Its structural formula is represented as CH_3COOH. Pure, water-free acetic acid (glacial acetic acid) is a colourless liquid that absorbs water from the environment (Togeas and James, 2005).

1.12.3. Fusidic acid

Fusidic acid is a bacteriostatic antibiotic that is often used topically in creams and eyedrops, but may also be given systemically as tablets or injections. Fusidic acid is only effective on Gram-positive bacteria such as *Staphylococcus spp*. and *Corynebacterium spp*.. Fusidic acid inhibits

bacterial replication and does not kill the bacteria and is therefore termed "bacteriostatic". Fusidic acid is often found in topical skin and eye preparations (Howden and Grayson, 2006).

1.12.4. Clotrimazole

Treatment of susceptible fungal infections including oropharyngeal candidiasis, dermatophytoses, superficial mycoses and cutaneous candidiasis. Avoid contact with eyes. Wash hands before applying or wear gloves. Apply thin film to affected area. May apply porous dressing. Report persistent burning, swelling, itching, worsening of condition, or lack of response to therapy (Duhm *et al.*, 1974).

1.13. High-Performance Liquid Chromatography (HPLC)

Since as early as the 1970's. HPLC was used for the determination of flavonoids and has become a popular method for separation, screening and quantitative analysis of plant, food products and also herbal medicines (Santos-Buelga *et al*., 2003; Liang *et al*., 2004; Wills and Stuart, 2004).

High reproducibility and accuracy were obtained by performing HPLC on different tea samples (Sharma *et al.*, 2005).

A simple gradient elution method efficiently separated various tea biochemicals (catechins, galic acid and xanthine alkoids) using acetonitrial and 0.1% ortho-phosphoric acid in water was used. HPLC has also been used to quantify the xanthone mangiferin (De Nysschen *et al*., 1996; Richards, 2002; Joubert *et al*., 2003; Botha, 2005; Van der Merwe, 2005), isomangiferin (Joubert *et al.,* 2003), hesperidin (Richards, 2002; Joubert *et al.,* 2003; Van der Merwe, 2005).

Separation systems for flavonoids in foods have been focused toward measurement of all (usually several subclasses) of prominent flavonoids (Merken and Beecher, 2000).

Not only can HPLC be widely used in quantification of various flavonoids and other phenolics, it can also easily be adapted for identification of individual compounds and therefore has the advantage of generating a chemical fingerprint used for the identity and quality of the given species. HPLC pattern of a standardised extract can also prevent the problem of adulteration due to misidentification of the herb in the field (Anonymous, 2006).

A new faster method has simulataneous analysis of the main flavonoid aglycones (flavanols, flavonols, flavanones, flavones) in food samples under 15 min. (Repollés *et al.*, 2006).

Chapter Two

Materials and Methods

2.1. Materials

2.1.1. Apparatus and equipments

The following equipments and apparatus were used throughout the experimental work:

Apparatus	**Company and origin**
Autoclave	Tony / Japan
Autoclave	Gallenkamp / England
Balance	Ohans / France
Compound Light Microscope	Olympus / Japan
Deep freezer	Sanyo / Japan
Distillator	GFL / Germany
Electric balance	Mettler / Switzerland
Electrical incubater	Gallenkamp / England
Electric oven	Gallenkamp / England
Filter papers	Halzfeld / Germany
Hot plate with magnetic stirrer	Gallenkamp / England
HPLC (High-Performance Liquid Chromatography)	Gallenkamp / England
Incubator	Gallenkamp / England
Laminar air flow cabinet	ESCO / Singapore
Micropipettes	Brand / Germany
Millipore filter	Millipore and Whatman / England
Millipore filter unit (0.22 µm)	Millipore and Whatman / England
Oven	Gallenkamp / England
pH-meter	Metter Gmbh-Teledo / England
Refrigerator	Concord / Iraq

Apparatus	**Company and origin**
Rotary evaporator	Gallenkamp / England
Sensitive balance	Delta Range / Switzerland
Shaker incubator	Sanyo / Japan
Soxhlet	Electrothermal / England
Vortex	Stuart Scientific Co. Ltd. / England
Water bath	Gallenkamp / England

2.1.2. Chemicals

The following chemicals were used during this study:

Chemicals	**Company and origin**
2, 4-dichlorophenoxyacetic acid(2,4-D)	BDH-England
Acetonitrile	GCC-England
Apigenin	Sigma-Germany
Azulene	Fluka-Germany
Benzyl adenine(BA)	BDH-England
Chloromphenicol.	Troge-Germany
Crystel violet	BDH-England
Ethanol absolute 99.5%	GCC-England
Ethanol 96%	Iraq
H_3PO_4	GCC-England
Iodine	BDH-England
Glycine	BDH-England
Methanol	GCC-England
$NaHPO_4$, NaH_2PO_4	BDH-England
NaOCl	Iraq

Chemicals	**Company and origin**
Safranin	BDH-England

2.1.3. Culture media

Medium	**Company and origin**
Agar - agar	Sleeze / England
Brain - Heart Infusion broth	Difco / USA
Nutrient broth	Oxide / England
Sabouraud Dextrose Agar (SDA)	Oxide / England
Tryptone soya agar	Oxide / England

2.1.3.1. Nutrient agar

These media were prepared as a recommended by the manufacturing companies; pH was adjusted to 7.0, autoclaved at 121°C for 15 min.

2.1.3.2. Murashige and Skoog, 1962 (MS) culture medium components

Chemicals	**Company and origin**
Ammonium nitrate (NH_4NO_3)	Fluka / Switzerland
Boric acid (H_3BO_3)	Merek / Germany
Calcium chloride anhydrate ($CaCl_2.2H_2O$)	Fluka / Switzerland
Cobalt chloride.$6H_2O$ ($CoCl_2.6H_2O$)	BDH / England
Cupric sulphate.$5H_2O$ ($CuSO_4.5H_2O$)	BDH / England
Ferrous sulfate.7 H_2O ($FeSO_4.7H_2O$)	BDH / England
Glycine	BDH / England
Magnesium sulphate anhydrate ($MgSO_4.7H_2O$)	Fluka / Switzerland

Chemicals	**Company and origin**
Manganese sulphate.$4H_2O$ ($MnSO_4.4H_2O$)	BDH / England
Molybdic acid (sodium salt).$2H_2O$ ($Na_2MoO_4.2H_2O$)	BDH / England
Myoinositol ($C_6H_6(OH)_6$)	BDH / England
Nicotinic acid ($C_8H_{11}NO_3.HCl$)	BDH / England
Potassium iodide (KI)	BDH / England
Potassium nitrate (KNO_3)	BDH / England
Potassium phosphate monobasic (KH_2PO_4)	Fluka / Switzerland
Pyrodoxine. HCl ($C_6H_5NO_2$)	BDH / England
Sodium ethylene diamine tetraacetate (Na_2-EDTA)	Fluka / Switzerland
Thiamine. HCl ($Cl_2H_{17}ClN_4OS$. HCl)	BDH / England
Zinc sulphate.$7H_2O$ ($ZnSO_4.7H_2O$)	BDH / England

2.1.4. Drugs

Generic name	**Trade name**	**Manufacturer**
Acetic acid 6% solution	Vinegar	Iraq
Cefotaxime sodium 1g powder	Claforan	Turkey – Bilim
Clotrimazol 1% solution	Candi – mazol	Syria – Domina
Fusidic acid 20g cream	Fucine	Syria – Ibn - Hayyan

2.1.5. Animals

Twenty four local albino mice (female), 4-6 weeks of age weighting 20-22g were used in the experiment, each cage was containing 3 mice. Animals were all subjected to 22-25°C for 14 /10 hrs. light/dark photoperiod. They were supplied by Biotechnology Dept., College of Science Al-Nahrain University, Iraq. Twenty four albino mice (female),

with similar characteristics and environmental conditions were obtained from Pharmacy College, Damascus University, Syria.

2.1.6. Microorganisms

The following microorganisms were obtained from Biotechnology Dept., College of Science, Al-Nahrain University: *Candida albicans, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

2.1.7. Buffer (Atlas *et al.*, 1995)

Phosphate buffer saline (PBS) pH 7.2 was prepared as follows:

- Solution A: A quantity of 13.9 g of sodium phosphate dihydrate (NaH_2PO_4) was dissolved in 1L of D. W.
- Solution B: A quantity of 17.79 g of sodium phosphate monohydrate ($NaHPO_4$) was dissolved in 1L of D. W.

The final solution was prepared by adding 13 ml of solution A to 87 ml of solution B then 100 ml of D. W. The final volume of PBS was 200 ml. This was sterilized by autoclaving at 121°C for 15 min.

2.1.8. Stains

Gram's Stain (Atlas *et al.*, 1995).

It composed of 4 reagents:

1- A primary stain - Crystal Violet.

2- A mordant - Gram's Iodine Solution.

3- A decolorizing agent-an organic solvent (alcohol 95%).

4- A secondary stain or counter stain - Safranin.

This stain was prepared by dissolving 2 g of crystal violet in 20 ml of 95% ethanol and the final volume was completed to 100 ml with D. W. and filtered by using filter paper (Whatman no.1) before use.

2.1.9. Plant material

Chamomile dried flowers were bought from local market and identified as a German chamomile by Prof. Dr. Ali H. Al-Mosawy, Department of Biology, College of Science, The University of Baghdad.

2.2. Methods

2.2.1. Preparation of culture media

2.2.1.1. Tryptone soya agar medium (Atlas *et al*., 1995)

Tryptone soya agar medium was prepared by dissolving 37g of tryptone soya agar in 1L of D. W. and sterilized by autoclaving at 121°C for 15 min.

2.2.1.2. Nutrient broth medium (Atlas *et al*., 1995)

It was prepared by dissolving 37g of nutrient broth in 1L of D. W. and sterilized by autoclaving at 121°C for 15 min. for bacterial culture.

2.2.1.3. Sabouraud Dextrose Agar (SDA) (Jong, 1981)

This medium was prepared by dissolving 10 mg of peptone, dextrose (20 mg) and agar (15 mg) in 100 ml D. W. The pH was adjusted to 7 and then sterilized by autoclaving and the medium was supplemented with 0.5 mg/ml chloromphenicol, for culturing *C. albicans.*

2.2.1.4. Preparation of plant tissue culture medium

Murashige and Skoog, 1962 (MS) medium was prepared and used (Table 1). Sucrose 35 g/l, myoinositol 0.1 g/l and the plant growth regulators (2, 4 -D and BA) at different concentrations were added. The pH was adjusted to 5.8 using NaOH or HCl (1N), and then 8 g/l of the agar type (Agar-Agar) was added to the medium, all dissolved in 1L D.W., placed on a hotplate magnetic stirrer till boiling, then aliquots of 20 ml were dispensed into (10×5) cm culture vessels.

Culture media were sterilized by autoclaving at 121°C under (15 Ib/in^2) pressure, for 15 min. The medium was left at room temperature to cool and became ready to culture explants**.**

Glassware and other instruments were either autoclaved or placed in an electric oven at 200°C for at least 2 hrs. (Cappuecino and Sherman, 1987).

Table 1: Murashige and Skoog, 1962 (MS) culture medium components

Components	**Chemical formula**	**Weight (mg/l)**
Macronutrients		
Ammonium nitrate	NH_4NO_3	1650
Potassium nitrate	KNO_3	1900
Calcium chloride anhydrate	$CaCl_2.2H_2O$	440
Magnesium sulphate anhydrate	$MgSO_4.7H_2O$	370
Potassium phosphate monobasic	KH_2PO_4	170
Micronutrients		
Boric acid	H_3BO_3	6.20
Potassium iodide	KI	0.83
Manganese sulphate.$4H_2O$	$MnSO_4.4H_2O$	22.30
Zinc sulphate.$7H_2O$	$ZnSO_4.7H_2O$	8.60
Molybdic acid (sodium salt).$2H_2O$	$Na_2MoO_4.2H_2O$	0.25
Cupric sulphate.$5H_2O$	$CuSO_4.5H_2O$	0.025
Cobalt chloride.$6H_2O$	$CoCl_2.6H_2O$	0.025
Chelated Iron		
Sodium ethylene diamine tetraacetate	Na_2-EDTA	33.6
Ferrous sulfate.7 H_2O	$FeSO_4.7H_2O$	27.8
Vitamins		
Thiamine.HCl (B1)	$Cl_2H_{17}ClN_4OS.$ HCl	0.1
Nicotinic acid (B3)	$C_8H_{11}NO_3.HCl$	0.5
Pyrodoxine.HCl (B6)	$C_6H_5NO_2$	0.5
Glycine(free base)	$C_2H_5NO_2$	2.0
Myoinositol	$C_6H_6(OH)_6$	100

2.2.2. Maintenance of isolates

2.2.2.1. Maintenance of bacterial isolates

Maintenance of bacterial isolates was performed according to Maniatis *et al.,* 1982 and Atlas *et al*., 1995 as follows:

2.2.2.1.1. Short- term storage

Colonies of bacteria were maintained for a period of 2-3 weeks on the surface of Tryptone soya agar medium. The plates were tightly wrapped with parafilm and stored at 4°C.

2.2.2.1.2. Medium - term storage

Bacterial isolates were maintained by streaking on slants of tryptone soy agar or nutrient agar medium for a period of a few (2-3) months. Such medium was prepared in screw - capped vials containing 10-15 ml of the medium. The isolates were streaked on these slant media and incubated at 37°C for 24 hrs. Thereafter, the slants were taken and wrapped with parafilm and stored at 4°C.

2.2.2.1.3. Long - term storage

Bacteria can be stored for one year in either nutrient broth or Brain-Heart infusion broth medium containing 15% glycerol at low temperature without significant loss of viability. This was done by adding 1.5 ml of sterilized glycerol to fresh preparation of bacterial growth in a small screw capped vials (Bejo bottles) with final volume of 10 ml and stored at -20°C.

2.2.2.2. Maintenance of *Candida* isolates

The isolates were inoculated on SDA slants containing 0.5 mg/ml chloromphenicol. After incubation at 37°C for 48 hrs, the slants placed at 4°C as stock cultures. These slants were recultured every three months (Oliver *et al.,* 1982).

2.2.3. Measurement of bacterial growth

Bacterial growth was monitored by Macfarlane tube No. 5 turbidity standard which is equivalent to bacterial concentration for inoculums 1.5 x 10^8 cells/ml.

2.2.4. Identification of bacterial and yeast isolates

2.2.4.1. Morphological and cultural characteristics of bacteria

Morphology of *S. aureus* and *P. aeruginosa* colonies was studied on tryptone soya agar medium. Color, shape, size and edge of colonies were recorded after 24 hrs. of incubation at 37°C (Atlas *et al.*, 1995).

2.2.4.2. Morphological and cultural characteristics of yeast

After cultivation of suspected isolates on SDA and incubation at 37°C, morphology of colonies was examined and described (Savage and Balish, 1971). Part of the colony was fixed on a glass slide, stained by Gram's stain, then examined under oil-immersion lens of a compound light microscope (Kreger-van, 1984).

2.3. Preparation of bacterial suspension (Atlas *et al.*, 1995)

A colony was picked up from the plated medium by using a sterilized loop. The inoculum was emulsified in 5 ml suspended medium by rubbing against the slide of the tube and mixed thoroughly with the water.

2.4. Preparation of flower extracts

2.4.1. Water extract (Swanston *et al.*, 1990)

Plant powdered material was macerated with D. W. in a ratio of (1:5 w/v). A quantity of 50g of the powder was mixed with 250 ml D. W. The mixture was left in a shaker incubator at 37 ºC for 24 hrs. The suspension was filtered through a filter of gauze to get rid of the large

particles then filtered through a filter paper (Whatman no.1). The filtrate was concentrated to 200mg/ml using a rotary evaporator at 40ºC, filter-sterilized using Millipore unit (0.22µm), kept frozen at a temperature of -20ºC till use.

2.4.2. Ethanol extract (Harborne, 1973)

A quantity of 50g of flowers powder was extracted with 250 ml of 75% ethanol by soxhlet apparatus for 6 hrs. at 40-60ºC. The suspension was filtered through a filter paper (Whatman no.1), and then the solvent was removed under reduced pressure by using rotary evaporator at 40ºC, filter-sterilized using Millipore unit (0.22µm). The crude solid extract (24g) was kept in a deep freeze until use.

2.5. Initiation of callus cultures

2.5.1. Sterilization of explants (Pierik, 1987)

Seeds were rinsed with tap water for 10 min. then transferred to the laminar air flow-cabinet where submerged in sodium hypochlorite at different concentrations (0.0, 1.5, 3.0 or 6.0)% for 5 or 10 min. Seeds then rinsed with sterilized D. W. for three times. For each concentration, 12 seeds were used and distributed into 4 culture jars.

2.5.2. Plant growth regulators (Zhao *et al*., 2001)

Different concentrations of the auxin 2,4-D (0.0, 0.1, 0.2 or 0.3) mg/l and the cytokinin BA (0.0, 0.5, 1.0, 2.0 or 3.0) mg/l were prepared and added to the culture media as required before autoclaving.

2.5.3. Incubation of cultures (Ramawat, 2008)

Surface sterilized seeds were transferred into the culture vessels under aseptic conditions, placed in the incubator at 25°C for 16/8 hrs. light/dark

photoperiod using day light inflorescents at light intensity of 1000 lux.

2.5.4. Induction of callus cultures (Ramawat, 2008)

Different combinations of plant growth regulators were examined to determine the most effective one for callus initiation. Seeds were placed onto MS medium containing 2,4-D and BA at the concentrations mentioned above. Cultures were placed in the incubator as stated in 2.5.3. The response of these explants to auxin and cytokinin combinations was evaluated after 21 days in culture.

2.5.5. Maintenance of callus cultures (Bos, 1997)

The initiated callus was removed from the explants (germinating seeds) using forceps and scalpel, and then pieces weighting approximately 50 mg were subcultured onto MS fresh medium supplemented with the same combinations of 2,4-D and BA. Callus fresh weight was determined using a sensitive balance then oven dried at 40°C for 24 hrs. for callus dry weight measurements.

2.6. Preparation of callus extracts for antimicrobial activity

Callus ethanol extracts that originally initiated on explants were prepared for antimicrobial activity as below:

2.6.1. Ethanol extraction (Harborne, 1973)

A quantity of 10g of callus powder was mixed with 50 ml of 75% ethanol then placed in soxhlet apparatus for 6 hrs. at (40-60) °C. The suspension was filtered through a filter paper (Whatman no.1), and then the solvent was removed under reduced pressure by using rotary evaporator at 40ºC, filter-sterilized using Millipore unit (0.22µm). The final concentration is 200mg/ml.

2.7. Determination of the antibacterial and antifungal activity (Nathan, 1978)

The activities of extracts were determined against target microorganisms (*S. aureus*, *P. aeruginosa* and *C. albicans*) *in vitro* by using modified agar diffusion method.

The flower water or ethanol extract solutions were collected as a solution and dried using a rotary evaporator at 40°C. The stock solution was prepared by dissolving 5 g of plant extract residue with 50 ml of sterile D. W. The extracts were prepared at different concentrations 10, 20 or 40 mg/ml. The dried water extract was redissolved with D. W. while the dried alcohol extract was redissolved with alcohol (75% ethanol).

The Tryptone soya agar medium was mixed well and aliquots of 20 ml were poured in Petri-dishes. The medium was inoculated with 0.1 ml of (1.5×10^8 CFU/ml) target isolates of *S. aureus*, *P. aeruginosa* or *C. albicans* by using sterile swabs.

Four evenly spaced wells, 3 mm in diameter were made in the agar of each plate with sterile cork borer. To identify the intrinsic extracts activity, one control well was filled with (100 µl) phosphate buffer saline. An equal volume of different concentrations 10, 20 or 40 mg/ml of the extracts was dispensed into each well (three replica plates were prepared for each agent). Test plates were then incubated at 37°C for 24 hrs. and zones of inhibition were measured using a ruler in millimeters.

A clear zone indicated that the extract showed its antibacterial or antifungal activity. This method was repeated three times for each test.

The activity of the drugs (Cefotaxime sodium, Fusidic acid, Clotrimazole and Acetic acid 6%) that used for treatment of skin

infection was investigated. The effect of flower and callus extracts of chamomile individually or combined with these drugs were determined by using modified agar diffusion method.

Commercially available 60 mm Petri dishes containing Tryptone Soya Agar were inoculated with 0.l ml of $1.5x10^8$ CFU/ml target isolates of *S. aureus*, *P. aeruginosa* or *C. albicans* by using sterile swabs. Six evenly spaced holes 3mm in diameter were made in the agar of each plate using sterile cork borer. To identify the intrinsic antibacterial drugs activity of the diluents, two control wells were filled with chamomile extract 40 mg/ml and drug alone 250mg/ml (100 µl), respectively. The tested wells were containing 50% (20mg/ml) of chamomile extract +50% of the drug (50%). An equal volume of each drug was expressed into each well, the other one contained 25% of the drug + 75% (30mg/ml) of chamomile extract (25%) while the other one contained 75% of the drug + 25% (10mg/ml) of chamomile extract (75%). Test plates were then incubated at 37°C for 24 hrs. and zones of inhibition were measured using a ruler in millimeter. A clear inhibition zone indicated that the agent had retained its antibacterial activity. All these procedures were performed on the callus ethanol extract.

2.8. Examination of flower extract activity on infected skin *in vivo* (Kugelberg *et al.*, 2005)

The *in vivo* study of possible therapeutic effect of chamomile extract on bacterial infections was performed on mice skin. The chamomile extracts were prepared and applied locally on the skin of experimentally infected mice.

In this study, two groups (A and B) of mice were used, each group included twelve mice and these were divided into four subgroups represented by three mice for each group. The experiment was

conducted as follows:

a- The hair on the back of mice was shaved, and the area was cleaned and disinfected with cotton swab saturated with 70% alcohol.

b- Direct scraping of the skin was done by sterile pathological scalpel to make abrasion on one half of the mouse's back skin.

c- After half an hour, the skin of each group was subjected to the infections as stated below (mice were treated with different treatments daily):

2.8.1. Group A: represented by twelve mice, this divided into four subgroups representing three mice for each. The scratched skin infected with 0.1 ml of *S. aureus* suspension that was concentrated with $1.5x10^8$ CFU/ml from an overnight *S. aureus* grown culture.

The signs of a wound infection exhibited redness, swelling, and pus appeared after 10 days.

2.8.1.1. The first subgroup: each of the three mice was smeared by a suspension of *S. aureus* and after ten days of the infection, the infected skin was treated with 0.1 ml (the drug Cefotaxime sodium 125mg/ml + chamomile flower ethanol extract 20mg/ml) at 50% from each. This group was used for infection and treatment of mice.

2.8.1.2. The second subgroup: each of the three mice was smeared by a suspension of *S. aureus* and after ten days of the infection, the infected skin was treated with 0.1 ml of the drug Cefotaxime sodium (250mg/ml).

2.8.1.3. The third subgroup: each of the three mice was smeared by a suspension of *S. aureus* and after ten days of the infection, the infected skin was treated with 0.1 ml of the chamomile flower ethanol extract (40mg/ml).

2.8.1.4. The fourth subgroup: each of the three mice was smeared by a suspension of *S. aureus* and after ten days of the infection, the infected skin was treated with 0.1 ml of PBS.

2.8.2. Group B: represented by twelve mice, which divided into four subgroups representing three mice for each. The scratched skin infected with 0.1 ml of *C. albicans* suspension that was concentrated with $1.5x10^8$ CFU/ml from *C. albicans* culture.

The signs of a wound infection appeared as redness, swelling, and pus after 10 days.

2.8.2.1. The first subgroup: each of the three mice was smeared by a suspension of *C. albicans* and after ten days of the infection, the infected skin was treated with 0.1 ml (the drug Clotrimazole 0.75mg/ml + chamomile flower ethanol extract 10mg/ml) at 75% from each. This group was used for infection and treatment of mice.

2.8.2.2. The Second subgroup: each of the three mice was smeared by a suspension of *C. albicans* and after ten days of the infection, the infected skin was treated with 0.1 ml of the drug Clotrimazole 1%.

2.8.2.3. The third subgroup: each of the three mice was smeared by a suspension of *C. albicans* and after ten day of the infection, the infected skin was treated with 0.1 ml of the chamomile flower ethanol extract (40mg/ml).

2.8.2.4. The fourth subgroup: each of the three mice was smeared by a suspension of *C. albicans* and after ten day of the infection, the infected skin was treated with 0.1 ml of PBS.

2.9. Administration of callus chamomile extract on infected skin *in vivo* (Kugelberg *et al.*, 2005)

The same procedure for flower extraction indicated in 2.8. was followed which was used for callus extract on infected skin but was carried out in the college of pharmacy at Damascus University, Syria.

2.10. Chromatographic procedure using HPLC

The method HPLC (High-Performance Liquid Chromatography) was carried out in the college of pharmacy at Damascus University, Syria.

2.10.1. Azulene (Adriana *et al*., 2004)

Azulene in ethanol extracts was estimated by gradient HPLC. Chromatography conditions: column Tessek SGX C18 7 μm (4 ¥ 250 mm); flow rate 0.7 ml·min1; mobile phase A: acetonitrile/water/H_3PO_4 (19:80:1), B: 45% acetonitrile, C: 90% acetonitrile. The linear gradient elution programme was from 100% A to 100% B in 25 min, then to 100% C in 30 min, isocratic for 5 min, and returning to 100% A in 45 min. Detection was performed at 320 nm of wavelength**.**

2.10.2. Apigenin (Benguo *et al*., 2008)

Apigenin standard was dissolved in methanol. A stock solution of 0.567 mg/ml was prepared. For HPLC calibration curve, a concentrations ranged from 0.0567 to 0.567 mg/ml were prepared.

The analytical method was used with some modifications. The samples were separated on a reversed phase column, Symmetry C18 column (3.9´ 150 mm; 5 mm particle size), manufactured by Waters, USA. The mobile phase consisted of methanol and water in a volume ratio of 50:50 with a flow rate of 0.8 ml/min. The HPLC system consisted of Waters 1525 Binary HPLC Pump and Waters 2487 Dual Wavelength Absorbance Detector. The injection volume was 10 ml and the detector was set at 259 nm. Before HPLC analysis, all samples had to be passed through a 0.45μm Millipore filter.

2.11. Statistical design and analysis (SAS, 2001)

A completely randomized design using factorial experiments were used to study the effect of BA and 2,4-D (two way analysis). Least significant differences (LSD) were calculated and means were compared. Twelve replicate explants were used for each treatment. For the determination of inhibition zone diameters, the mean of 3 replicates was calculated and the standard deviation was recorded.

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Chapter Three

Results and Discussion

3.1. Surface sterilization of explants (seeds)

The results in figure 4 showed that the most effective concentration of NaOCl was 3% for 10 min. that gave the highest percent (100%) survival. Increasing in concentration of NaOCl to 6% caused serious reduction in survival rate, whereas lowering the concentration led to a high rate of contamination.

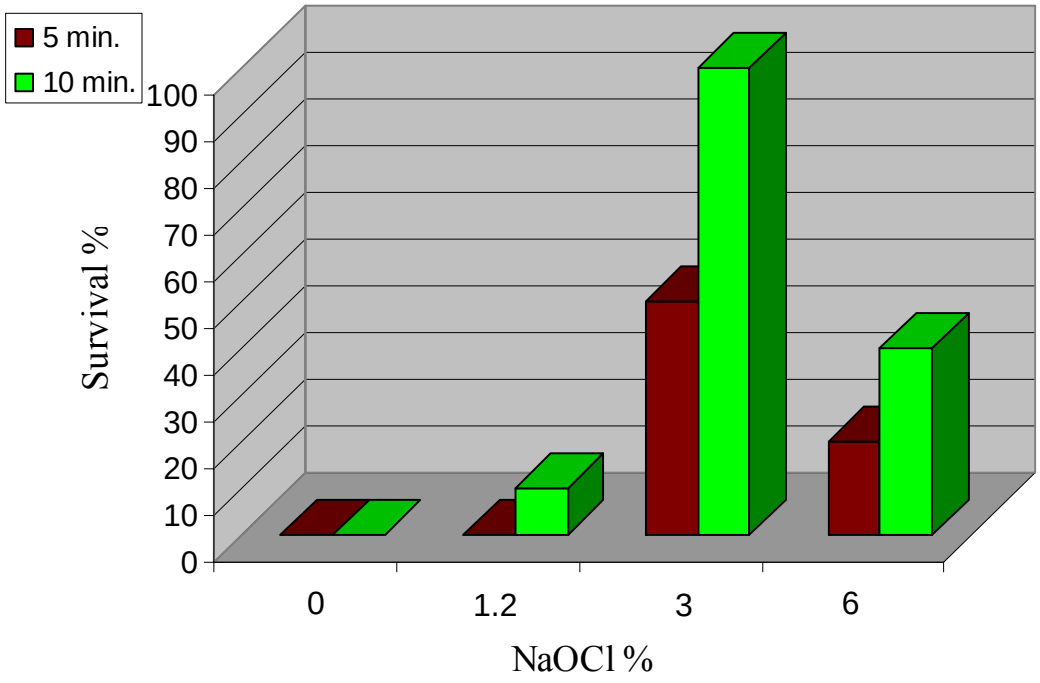

Fig. 4: Effect of different concentrations of NaOCl on explants survival at sterilization periods of 5 or 10 min.

Treatment of explants with NaOCl was important to eliminate the contaminants. It is used widely for plant tissue sterilization. The selection of sterilizing material depends on the source of explant, roughness of its surface and other factors. The sterilization material should be easy to remove from explants when washed with sterilized DH_2O (Yeoman and Macleod, 1977; Sateesh, 2003).

Pierik, (1987) referred to the importance of NaOCl for explants sterilization. Increase the surface sterilization period and concentration often lead to serious reduction in survival rate. Optimization experiment is therefore necessary to achieve maximum survival rate with a minimum contamination.

The harmful effect of NaOCl to explants is due to the presence of hypochlorous acid (HOCl). This is a strong oxidation material. The acid (HOCl) results from dissolving the chloride in water (as shown in the equation) are responsible for the surface sterilization of the explants (Ramawat, 2008).

$$Cl_2 + H_2O = HCl + HOCl$$

3.2. Induction of callus cultures

The effect of different concentrations of 2,4-D and BA on the response (%) for callus induction on explants is shown in Table 2.

Table 2: Effect of different concentrations of 2,4-D and BA on the response (%) of callus induction on chamomile seed explants.

2,4-D (mg/l)	BA (mg/l)				Mean
	0.0	0.5	1.0	2.0	
0.0	0.00	14.44	24.00	28.10	16.63
0.1	7.11	79.20	100.00	82.21	67.13
0.5	13.90	70.10	75.00	62.20	55.3
1.0	20.22	25.20	27.20	0.00	18.15
Mean	10.27	47.23	56.55	43.10	
LSD ≤ 0.05	BA=1.667		2,4-D=1.667	BA × 2,4-D=3.334	

n= number of replicates, (n=12).

All concentrations of BA led to a significant increase in the percentages of explants led to callus induction (47.23, 56.55 and 43.10%) at the concentrations 0.5, 1.0 and 2.0 mg/l, respectively, compared with the control treatment (10.27%).

Addition of 2,4-D at 0.1 mg/l led to a significant increase in the percentage of explants showed callus induction reaching (67.13%) compared with the control whereas only 16.63% of explants initiated callus. Percentage of explants that initiated callus decreased when 2,4-D was added at 0.5 mg/l and 1.0 mg/l reached 55.3 and 18.15% respectively, however, they still significantly higher than those untreated.

The interaction between the two growth regulators achieved 100% response in a combination of 1.0 mg/l BA and 0.1 mg/l 2,4-D (Fig. 5). The response decreased to 82.21% when BA concentration was increased to 2.0 mg/l in the presence of 0.1 mg/l 2,4-D. While no callus induction was reported on untreated explants and the combination of 2.0 mg/l of BA and 1.0 mg/l of 2,4-D. Chamomile seedlings may contain some levels of endogenous auxins that made a balanced ratio with the exogenous cytokinin resulting in an effective combination for callus induction.

Callus induction requires a balanced ratio of auxin(s) and cytokinin(s) as stated by Skoog and Miller (1957). In a number of plant species, callus induction favors higher auxins than cytokinins, but Murashige and Skoog, 1962 stated that seed is a rich source of auxins so that a little amount of 2,4-D was required for callus initiation (Ramawat, 2008).

Establishment of a callus tissue on explants was illustrated by Dodds and Roberts (1995) who divided the process into three developmental stages: induction, cell division and differentiation. The

length of these phases depends mainly on the physiological status of the explant cells as well as the cultural conditions including the appropriate combination of plant growth regulators.

Fig. 5: Callus induction on seedling explants of chamomile grown on MS medium containing a combination of 1.0 mg/l BA and 0.1 mg/l 2,4-D, after 21 days of culturing.

3.3. Maintenance of callus cultures

All concentrations of BA led to a significant increased in callus fresh weight (400.5, 756.25 and 416.25 mg) at the concentrations 0.5, 1.0 and 2.0 mg/l, respectively, (Table 3) compared with those not supplemented with BA (78.07 mg).

Addition of 2,4-D at (0.1 or 0.5mg/l) caused a significant increased in callus fresh weight (848.8 and 521.77mg), respectively, compared with 2,4-D free cultures (125.5mg). However, callus fresh weight decreased significantly when the concentration of 2,4-D increased to 1.0 mg/l

reaching to 155mg compared with those treated with 0.1 or 0.5 mg/l, although it was still significantly different from cultures not supplemented with 2,4-D (125.5)mg.

Table 3: Effect of different concentrations of 2,4-D and BA on callus fresh weight (mg) initiated on chamomile explants.

2,4-D (mg/l)	BA (mg/l)				Mean
	0.0	0.5	1.0	2.0	
0.0	0.00	102	225	175	125.5
0.1	95.20	700	1750	850	848.8
0.5	97.10	650	700	640	521.77
1.0	120	150	350	0.00	155
Mean	78.07	400.5	756.25	416.25	
LSD ≤ 0.05	BA=11.474		2,4-D=11.474		BA × 2,4-D=22.948

n = number of replicates, (n=12).

The interaction between the two growth regulators resulted in a maximum callus production reached (1750mg) at the combination 1.0 mg/l and 0.1 mg/l of BA and 2,4-D, respectively (Fig 6).

All combinations were significantly higher than those lacking both growth regulators except the combination 2.0 mg/l of BA and 1.0mg/l of 2,4-D since both showed deterioration and death of callus.

The highest dry weights of calli (Table 4) were exhibited in the combination of 1.0 mg/l of BA and 0.1mg/l of 2,4-D reached 150mg which was significantly higher than all other treatments.

Fig. 6: Callus cultures initiated on explants cultured on MS medium containing 1.0 mg/l BA and 0.1 mg/l 2,4-D, after 28 days of culturing.

Table 4: Effect of different concentrations of 2,4-D and BA on callus dry weight (mg) initiated on chamomile explants.

2,4-D (mg/l)	BA (mg/l)				Mean
	0.0	0.5	1.0	2.0	
0.0	0.00	8.50	11.50	40.10	15.02
0.1	7.50	60.50	150.00	70.20	72.05
0.5	8.00	50.40	52.20	51.30	40.47
1.0	6.50	30.90	30.00	0.00	16.85
Mean	5.50	37.57	60.925	40.40	
LSD ≤ 0.05	BA=2.498		2,4-D=2.498		BA × 2,4-D=4.997

n = number of replicates, (n=12).

According to the results stated above, callus was induced on germinating seedling explants then maintained for many subcultures on MS medium containing 1.0 mg/l BA and 0.1 mg/l 2,4-D for subsequent experiments.

Increasing the levels of the two plant growth regulators suppressed callus growth. The increase of callus mass is important as a source for the production of secondary metabolites since they are proportionally related (Ramawat, 2008).

Shoots appeared from callus cultures were noticed when some calli left on the maintenance medium for 60 days without subculture as shown in Table 5 and Fig 7.

Table 5: Shoot regeneration from chamomile callus cultures grown on MS medium supplemented with BA and 2,4-D for 60 days.

2,4-D (mg/l)	BA (mg/l)			
	0.0	0.5	1.0	2.0
0.0	-	-	+	+
0.1	-	-	+	+
0.5	-	-	-	-
1.0	-	-	-	-

+ : Regenerated shoots from callus cultures

- : no regeneration was recorded

Shoots were regenerated from callus at the combination of 1.0 or 2.0 mg/l of BA and 0.0 or 0.1 mg/l of 2,4-D. No shoot regeneration was reported at other treatments.

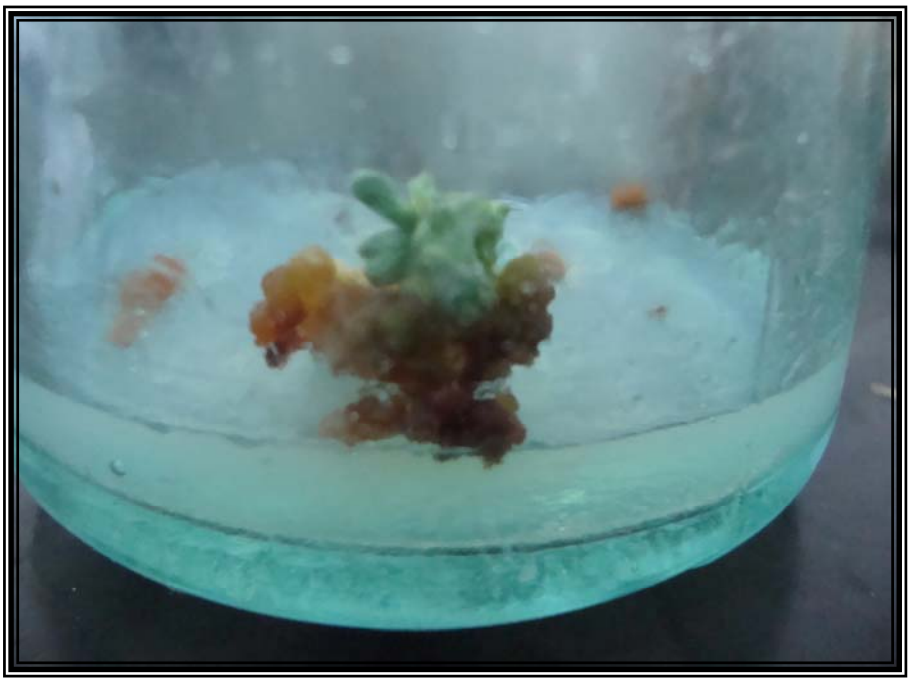

Fig. 7: Chamomile shoots regenerated from callus cultures after 60 days grown on MS medium supplemented with 2.0 mg/l of BA and 0.1 mg/l of 2,4-D.

3.4. Characters of chamomile flower and callus extracts

A brownish, clear liquid with an intense characteristic odour and bitter taste; miscible with water and soluble in ethanol.

3.5. Determination of the antibacterial and antifungal activity of extracts *in vitro*

Water and ethanol extracts obtained from dried flowers were investigated for their antimicrobial activity against *S. aureus, P. aeruginosa* and *C. albicans*.

3.5.1. Effect of water extract

Results displayed in Table 6 indicated that all concentrations of chamomile flowers water extracts have inhibitory effect against the Gram-positive bacteria *S. aureus* causing an inhibition zones with diameters of 12.4, 14.3 and 18.1 mm at the concentrations 10, 20 or 40 mg/ml, respectively. For *P. aeruginosa*, showed no inhibition in the control (PBS) treatments, slight inhibition at 10 and 20 mg/ml while caused an inhibition zone with 15.2 mm at the concentration 40 mg/ml.

Table 6: Diameter of inhibition zones caused by chamomile flower water extracts at various concentrations on *S. aureus*, *P. aeruginosa* and *C. albicans*

Concentration mg dwt /ml	**Diameter of inhibition zone (mm) ± S.D.**		
	S. aureus	***P. aeruginosa***	***C. albicans***
Control (PBS)	-ve	-ve	-ve
10	12.4±0.10	Slight inhibition	slight inhibition
20	14.3±0.30	Slight inhibition	13.3±1.04
40	18.1±0.31	15.2±0.10	15.1±0.20

- ve: no activity was observed.

Values are mean of 3 replicates ± S.D.

The inhibition ability was more pronounced against Gram-positive bacteria *S. aureus*, while it was less against the Gram-negative bacteria *P. aeruginosa.* In *C. albicans*, slight inhibition was observed at the concentration 10 mg/ml. While the concentrations at 20 or 40 mg/ml showed 13.3 and 15.1 mm inhibition zones, respectively.

These results were in agreement by with those of Korting and Schafer-Corting, 1993 who regarded G+ve bacteria especially *B. subtilis* and *S. aureus* are sensitive to chamomile flowers water extract, than G-ve bacteria *E. coli* and *P. aeruginosa* which relatively less sensitive. The antibacterial effect may depend on the concentrations of chamazulene, bisabolol and bisabolol oxides (A and B) in the extract. Even at concentrations, lower than 100µg/ml. a-bisabolol and its spiro-ether derivative were recorded as effective antibacterial agents.

Smith, (2006) referred that chamomile has antibacterial constituents such as chamazulene, bisabolol and bisabolol oxides. The resistance of G-ve bacteria could be due to the permeability barrier provided by cell membrane (Adwan and Abu-Hasan, 1998).

The results were also in accordance with Mann and Staba (1986) who showed the role of chamomile action in preventing infections and promotion of wound healing by fighting infection caused by *S. aureus*.

3.5.2. Effect of ethanol extract

Chamomile flowers ethanol extract exhibited antibacterial activity against microorganisms at the concentration 10mg/ml (table 7). The diameter of the inhibition zones against *S. aureus* was 12.1 mm at concentration of 10 mg/ml. The higher concentrations of extracts 20 and 40 mg/ml, *S. aureus* showed 13.2 mm and 15.0 mm, respectively. Results also showed that *P. aeruginosa* gave 29.7 mm inhibition zones at 10 mg/ml. While the concentrations of extracts showed 33.2 mm at 20 mg/ml and 37.4 mm at 40 mg/ml of the chamomile flowers ethanol extract.

In *Candida*, the low concentration 10 mg/ml showed 21.1 mm inhibition zones subsequently, 28.1 and 30.4 mm were recorded at the concentrations of 20 and 40 mg/ml.

Table 7: Diameter of inhibition zones caused by chamomile flowers ethanol extracts at various concentrations on *S. aureus*, *P. aeruginosa* and *C. albicans*

Concentration mg dwt /ml	**Diameter of inhibition zone (mm) ± S.D.**		
	S. aureus	***P. aeruginosa***	***C. albicans***
Control (PBS)	-ve	-ve	-ve
10	12.1±0.14	29.7±0.25	21.1±0.07
20	13.2±0.12	33.2±0.68	28.1±0.46
40	15.0±0.10	37.4±0.10	30.4±0.07

-ve: no activity was observed

Values are mean of 3 replicates ± S.D.

These results were in agreement with Al-naymi, (2005) who reported that ethanol extracts of chamomile flowers have higher activity than water ones, suggesting that the activity of chamomile could be attributed to the existence of chamazulene, α- bisabolol (sesquiterpenes) that showed high inhibition activity against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Micrococcuse ssp.* and *C. albicans.*

3.6. Determination of the antimicrobial activity of drugs and chamomile flower extracts

Results in table 8 indicate the *in vitro* effect of chamomile water extract on the antibacterial activity of some drugs and treatments that used in curing skin wounds. Cefotaxime sodium:water extract at 50% (125:20)mg/ml achieved the highest diameter of inhibition zone (65.0 mm) against *S. aureus*, but the Cefotaxime sodium:water extract at 75% (187.3:10)mg/ml gave inhibition zone of 59.3 mm against *P. aeruginosa*

and Cefotaxime sodium at 100% (250mg/ml) achieved the highest diameter of inhibition zone of 40.0 mm against *C. albicans*.

Table 8: Antimicrobial activity of chamomile flower water extracts 40mg/ml in combination with some antimicrobial drugs *in vitro*

Drugs (%)	**Final concentrations (mg/ml)**	**Diameter of inhibition zone (mm) ± S.D.**		
		S. aureus	***P. aeruginosa***	***C. albicans***
Cefotaxime sodium:extract				
25:75	62.5:30	64.0±1.00	50.1±2.10	37.0±2.00
50:50	125:20	65.0±1.00	52.2±0.17	38.0±2.00
75:25	187.5:10	60.2±1.00	59.3±1.51	39.5±4.10
100:0	250:0	48.1±0.95	48.1±0.90	40.0±1.00
0:100	Water extract 40	21.2±1.00	20.3±0.57	30.0±3.00
Acetic acid 6%:extract				
25:75	1.5:30	16.1±1.90	19.0±1.00	20.0±2.00
50:50	3:20	17.3±2.65	17.0±1.00	12.3±0.57
75:25	4.5:10	15.1±0.95	11.0±0.01	19.0±1.00
100:0	6%:0	17.5±1.10	18.5±1.00	21.1±0.01
0:100	Water extract 40	21.2±1.00	20.3±0.57	30.0±3.00
Fusidic acid:extract				
25:75	5000:30	25.0±0.95	-	21.0±1.00
50:50	10000:20	30.2±1.80	-	22.1±1.04
75:25	15000:10	27.0±2.00	-	24.0±0.02
100:0	20000:0	29.0±2.00	-	30.0±2.00
0:100	Water extract 40	21.2±1.00	20.3±0.57	30.0±3.00
Clotrimazole:extract				
25:75	0.25:30	*	*	24.1±1.00
50:50	0.50:20	*	*	26.0±1.00
75:25	0.75:10	*	*	27.0±1.00
100:0	1%:0	*	*	20.1±0.11
0:100	Water extract 40	*	*	30.0±3.00

Values are mean of 3 replicates ± S.D.

* Not tested since Clotrimazole is a fungicide

- No inhibition zone

Acetic acid at 6% alone achieved the highest diameter of inhibition zone (17.5 mm) against *S. aureus*, but Acetic acid:water extract at 25% (1.5:30)mg/ml recorded an inhibition zone of 19.0 mm against *P. aeruginosa* but the treatment with water extract only was better than Acetic acid only since it gave 20.3 mm inhibition zone against *P. aeruginosa*. The Acetic acid only however, achieved a diameter of inhibition zone recording 17.5 mm against *S. aureus*. Water extract alone achieved the largest inhibition zone reached 30.0 mm against *C. albicans*.

Fusidic acid at 50% (10000:20)mg/ml achieved the highest diameter of inhibition zone (30.2 mm) against *S. aureus* but no inhibition zone against *P. aeruginosa* was observed at all concentrations. Fusidic acid at 100% (20000mg/ml) gave an inhibition zone of 30.0 mm against *C. albicans*.

Clotrimazole was tested against *C. albicans* only, at 75% (0.75:10)mg/ml achieving a diameter of inhibition zone 27.0 mm compared with water extract (40mg/ml) which gave 30.0 mm of inhibition zone.

It is clear that Cefotaxime sodium was the most effective agent in inhibiting *S. aureus* and *P. aeruginosa* growth, but the most effective agent against *C. albicans* was Clotrimazole solution.

Cefotaxime sodium has the greatest inhibitory effect against tested microorganisms because it inhibits bacterial cell wall synthesis by binding to one or more of the Penicillin-Binding Proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested (Gilbert *et al*., 2007).

Clotrimazole showed moderate inhibitory effect against *C. albicans* since it binds to phospholipids in the fungal cell membrane altering cell wall permeability resulting in loss of essential intracellular elements (Hughes and Kriedman, 1984).

Results demonstrated that Cefotaxime sodium and the active compounds diluted with chamomile water extract produced inhibition zones larger than that resulted from undiluted agents or water extract only.

This may be due to the absorption of Cefotaxime sodium by agar (*in vitro*) during the treatment, and its ability had increased by using chamomile water extract **(**Gilbert *et al*., 2007).

Clotrimazole mixed with chamomile extract exhibited an increased inhibition zone only for *C. albicans*. Any antimicrobial agents used for burn or wound treatment should be tested in some manner for its ability to penetrate the skin.

Staphylococcins have some limitations, which reduce their effectiveness as drugs treatment for wounds and burns infections. First, they are not effective against most or some Gram-negative bacteria. Second, they are not effective against some Gram-positive bacteria and even in sensitive Gram-positive strains; there are sensitive variant cells which can multiply in the presence of a bacteriocin. Mary *et al.,* 1993 described that bacteriocins can be combined to have a better antibacterial effect against Gram-positive bacteria.

This study reports that chamomile water extract and some antibacterial agents; can be combined to have a better activity against *S. aureus*. The variation in activity may be due to the difference in adsorption for both chamomile water extract and drugs.

Drugs may be associated with the mechanism by which chamomile extract enters the cell after binding to the cell surface. It has been proposed that following of binding chamomile extract to the surface, the barrier functions of the cell wall of sensitive cells are impaired. This disruption of the cell wall function allows other molecules (drugs) to pass through the wall, come in contact with the cytoplasm membrane and destabilize its function (Ray and Daeschel, 1992).

Table (9) showed the *in vitro* effect of chamomile ethanol extract on the antibacterial activity of some drugs and treatments that used for curing skin of wounds. Cefotaxime sodium:ethanol extract 50% recorded the highest diameter of inhibition zone (70.0 mm) against *S. aureus*, but Cefotaxime sodium:ethanol extract 75% (187.5:10)mg/ml recorded an inhibition zone reached 61.0 mm against *P. aeruginosa* while Cefotaxime sodium:ethanol extract at 25% (62.5:30)mg/ml achieved the highest diameter of inhibition zone (32.1) mm against *C. albicans*.

Acetic acid at 100% achieved the highest diameter of inhibition zone (22.0 mm) against *S. aureus*, but Acetic acid at 25% (1.5:30)mg/ml gave an inhibition zone 13.2 mm against *P. aeruginosa* but the ethanol extract was better than Acetic acid in a diameter of inhibition zone (29.7 mm). The Acetic acid 75% (4.5:10)mg/ml achieved the diameter of inhibition zone (29.0 mm) while ethanol extract alone produced the largest inhibition zone (33.5 mm) against *C. albicans*.

Fusidic acid at 100% achieved the highest diameter of inhibition zone (40.5) against *S. aureus*, but no inhibition zone against *P. aeruginosa*, while giving an inhibition zone 30.5 mm against *C. albicans*.

Clotrimazole was tested against *C. albicans* only. At percentage 75% (0.75:10)mg/ml achieved a diameter of inhibition zone reached 24.3 mm compared with the ethanol extract which recorded 33.5 mm

inhibition zone and with the Clotrimazole at 100% which recorded 28.4 mm.

Table 9: Antimicrobial activity expressed as a diameter of inhibition zone caused by chamomile flower ethanol extract in combination with some antimicrobial drugs i*n vitro*.

Drugs (%)	**Final concentrations (mg/ml)**	**Diameter of inhibition zone (mm) ± S.D.**		
		S. aureus	***P. aeruginosa***	***C. albicans***
Cefotaxime sodium:extract				
25:75	62.5:30	60.1±1.05	48.0±1.05	32.1±1.00
50:50	125:20	70.0±1.00	50.2±2.00	31.0±1.00
75:25	187.5:10	65.1±0.95	61.0±1.00	28.3±0.20
100:0	250:0	46.0±1.00	48.1±1.00	36.2±0.95
0:100	alcohol extract 40	30.1±1.00	29.7±1.00	33.5±2.00
Acetic acid 6%:extract				
25:75	1.5:30	18.1±1.00	13.2±0.86	22.5±1.00
50:50	3:20	19.0±1.00	15.5±1.00	27.1±1.00
75:25	4.5:10	20.1±1.10	19.0±1.00	29.0±2.00
100:0	6%:0	22.0±1.00	24.0±1.00	20.0±1.00
0:100	alcohol extract 40	30.1±1.00	29.7±1.00	33.5±2.00
Fusidic acid:extract				
25:75	5000:30	36.0±1.00	-	32.1±1.00
50:50	10000:20	39.1±1.00	-	40.2±1.10
75:25	15000:10	37.2±1.00	-	37.2±1.00
100:0	20000:0	40.5±1.00	-	30.5±1.00
0:100	alcohol extract 40	30.1±1.00	29.7±1.00	33.5±2.00
Clotrimazole:extract				
25:75	0.25:30	*	*	24.0±1.00
50:50	0.50:20	*	*	21.1±1.00
75:25	0.75:10	*	*	24.3±1.00
100:0	1%:0	*	*	28.4±1.00
0:100	alcohol extract 40	*	*	33.5±2.00

Values are mean of 3 replicates ± S.D.

* Not tested since Clotrimazole is a fungicide

- No inhibition zone

From these results, Cefotaxime sodium has the highest inhibition on *S. aureus* and *P. aeruginosa*, but the most effective treatment against *C. albicans* was Clotrimazole solution.

It appears that ethanol extract is more efficient than water extract. The reason may be due to the compounds already extracted by ethanol particularly flavanoids (Appendix 1). These results were agreed with Al-naymi, (2005) who reported that ethanol extracts of chamomile flowers have higher activity than water ones.

3.7. The effect of flower extracts combined with drugs on mice skin infection *in vivo*

The ethanol extract of chamomile at a concentration of 40 mg/ml and drugs (Cefotaxime sodium and Clotrimazole) showed an obvious destroying effect on *S. aureus* and *C. albicans* in experimentally induced skin infection in mice compared to controls (infection). The infection showed swallowing, redness and filled with pus cells as shown in Fig. 8 and 9 which represents 2 groups of mice, group A was infected with *S. aureus*, and group B was infected with *C. albicans*.

The first subgroup was treated with (Cefotaxime sodium and chamomile flower ethanol extracts) at 50% (125:20)mg/ml. Mice skin cured after 16 days. The second subgroup was treated with Cefotaxime sodium. Mice skin cured after 17 days of treatment. The third subgroup was treated with chamomile flower ethanol extracts. Mice skin cured after 22 days of treatment. The fourth subgroup was treated with PBS. Mice were died after 12 days of infection, as in Fig. 8.

The results showed that the better treatment is (Cefotaxime sodium and chamomile ethanol extracts) at 50%, as in table 10.

Table 10: Effect of different treatments on curing mice skin infected with *S. aureus* for the four experimental mice subgroups

Sub grouping of experimental mice (group A)	**Types of treatment**	**Results**
First subgroup	(Cefotaxime sodium and chamomile flower ethanol extracts) at 50%	Complete cure after 16 days
Second subgroup	Cefotaxime sodium	Complete cure after 17 days
Third subgroup	Chamomile flower ethanol extracts	Complete cure after 22 days
Fourth subgroup	PBS	Died after 12 days

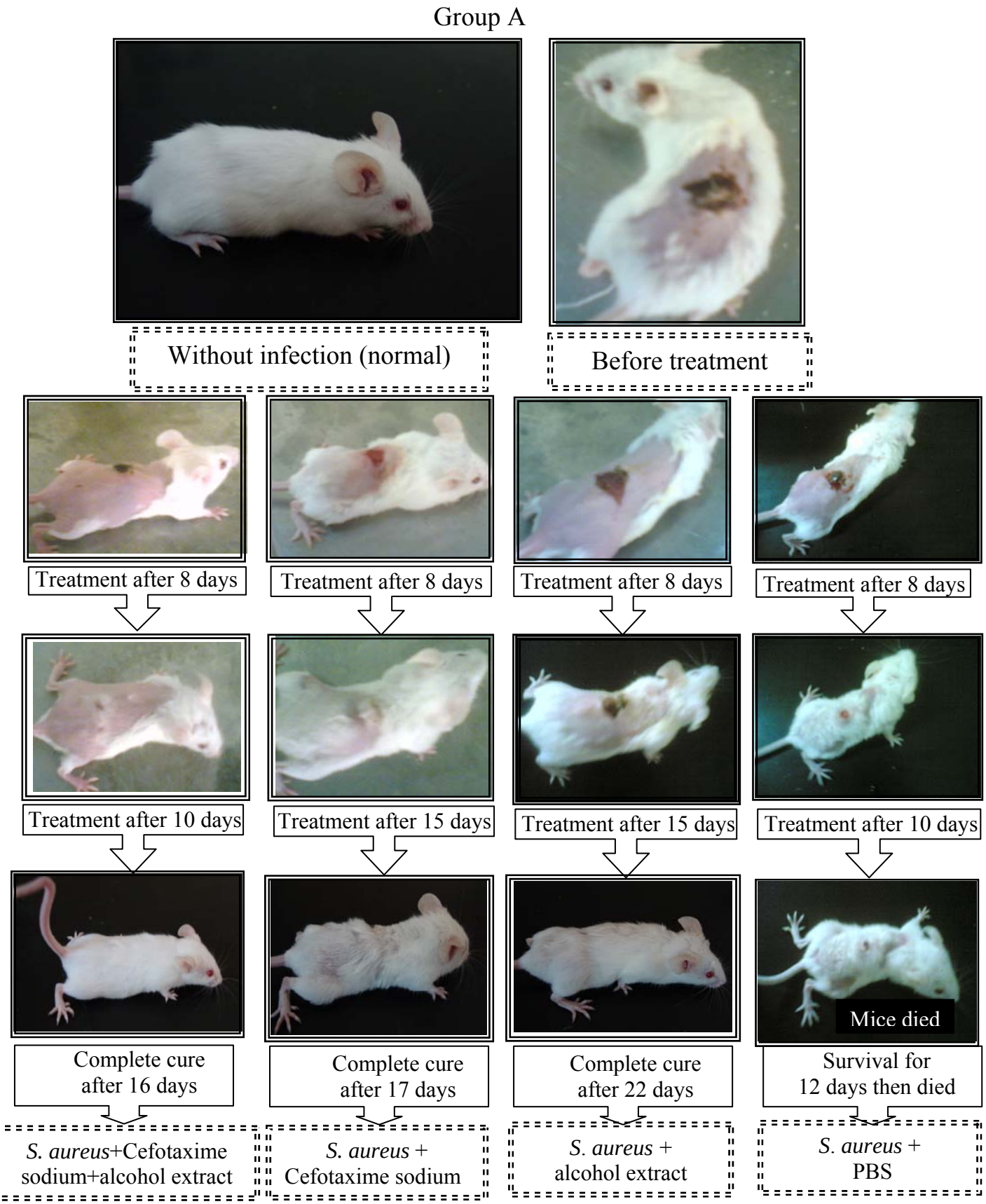

Fig. 8: Morphological repair of the skin infected by *S. aureus* and treated with a combination of Cefotaxime sodium + chamomile flower alcohol extract at a percentage 50%, Cefotaxime sodium, chamomile flower alcohol extract and PBS.

The group B contains four subgroups was infected with *C. albicans*. The first subgroup was treated with (Clotrimazole and chamomile flower ethanol extracts) at a percentage 75%. Mice skin cured after 20 days of treatment. The second subgroup was treated with Clotrimazole, Mice skin cured after 25 days of treatment. The third subgroup was treated with chamomile flower ethanol extracts. Mice skin cured after 10 days of treatment. The fourth subgroup was treated with PBS. Mice died after 8 days of infection as in Fig. 9.

The results indicated that the better treatment is chamomile flower ethanol extracts as in Table 11.

Merfort and Heilman (1994) conducted *in vivo* study on nine female volunteers. They stated that chamomile (that contains flavons, apigenin, luteolin and apigenin glycoside) absorbed at the skin surface and penetrated into deeper skin layers. This observation supports that use of chamomile as topical anti-inflammatory agents to treat inflammations in deep tissues such as cornea.

The effect of alcoholic extract of chamomile was tested in mice skin. It has an anti-inflammatory activity. The response was observed when chamomile extract was used to reduce swelling. Both bisabolol and the flavonoids are nearly active in reducing inflammations in animals (Joe and Teresa, 1999).

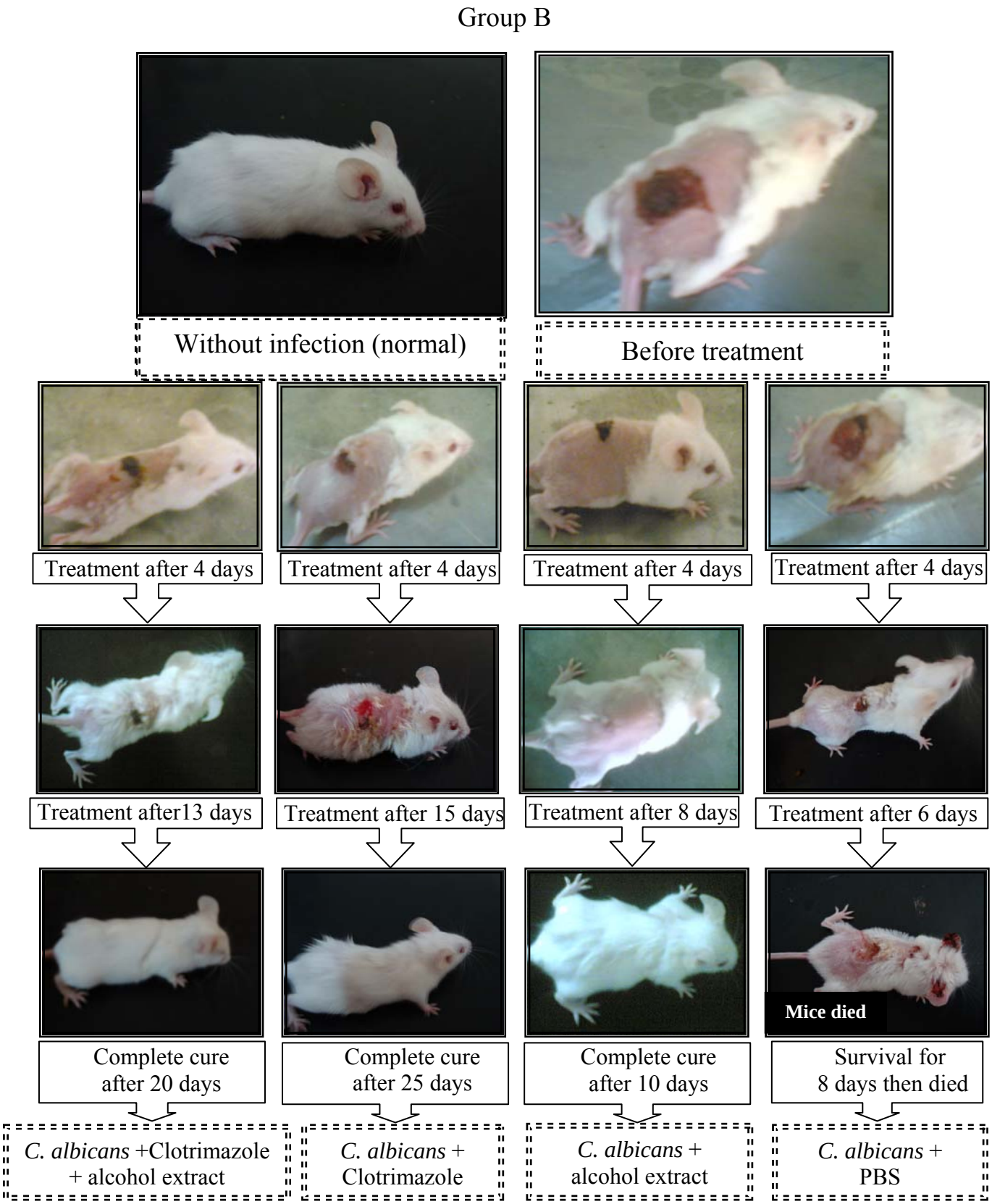

Fig. 9: Morphological repair of the skin infected by *C. albicans* and treated with a combination of Clotrimazole + chamomile flower alcohol extract at a percentage 75%, Clotrimazole, chamomile flower alcohol extract and PBS.

Table 11: Effect of different treatments on curing mice skin infected with *C. albicans* for the four experimental mice subgroups.

Sub grouping of experimental mice (group B)	**Types of treatment**	**Results**
First subgroup	(Clotrimazole and chamomile flower ethanol extracts) at a percentage 75%	Complete cure after 20 days
Second subgroup	Clotrimazole	Complete cure after 25 days
Third subgroup	Chamomile flower ethanol extracts	Complete cure after 10 days
Fourth subgroup	PBS	Died after 8 days

3.8. Determination of the antimicrobial activity of drugs and callus chamomile extract *in vitro*

Table (12) shows *in vitro* effect of the callus ethanol extract and antimicrobial drugs. Cefotaxime sodium was the most inhibiting drug against *S. aureus*, *P. aeruginosa* and *C. albicans*. Fusidic acid was the second in its effectiveness in inhibiting *S. aureus*, *P. aeruginosa* and *C. albicans* growth, while Acetic acid was the third. Clotrimazole was the most effective in inhibiting *C. albicans* growth.

Callus ethanol extract was more effective than chamomile ethanol flower extract in inhibiting *S. aureus*, *P. aeruginosa* and *C. albicans* growth.

Table 12: Antimicrobial activity expressed as the diameter of inhibition zone caused by callus cultures extracted with ethanol in combination with some in combination with some antimicrobial drugs *in vitro*.

Drugs (%)	**Final concentrations (mg/ml)**	**Diameter of inhibition zone (mm) ± S.D.**		
		S. aureus	***P. aeruginosa***	***C. albicans***
Cefotaxime sodium:extract				
25:75	62.5:30	69.1±2.00	60.0±4.00	50.1±0.11
50:50	125:20	80.0±5.00	60.1±1.00	53.1±0.11
75:25	187.5:10	76.2±2.05	70.0±5.00	70.0±4.00
100:0	250:0	70.0±4.00	68.0±2.00	48.5±1.80
0:100	Callus extract 40	35.5±5.00	38.7±1.00	45.0±2.00
Acetic acid 6%:extract				
25:75	1.5:30	20.1±2.00	18.1±2.00	25.1±1.00
50:50	3:20	21.1±1.00	16.0±1.00	28.3±2.51
75:25	4.5:10	24.0±2.00	20.1±1.00	30.1±1.00
100:0	6%:0	22.0±2.00	25.2±4.05	22.0±1.00
0:100	Callus extract 40	35.5±5.00	38.7±1.00	45.0±2.00
Fusidic acid:extract				
25:75	5000:30	33.0±1.52	30.1±4.00	33.1±2.00
50:50	10000:20	34.0±2.00	28.0±2.01	35.2±5.00
75:25	15000:10	35.0±2.05	31.0±3.00	36.3±2.00
100:0	20000:0	39.7±1.00	20.0±2.00	35.0±1.00
0:100	Callus extract 40	35.5±5.00	38.7±1.00	45.0±2.00
Clotrimazole:extract				
25:75	0.25:30	*	*	30.0±5.00
50:50	0.50:20	*	*	35.2±3.00
75:25	0.75:10	*	*	40.1±4.00
100:0	1%:0	*	*	30.1±2.00
0:100	Callus extract 40	*	*	45.0±2.00

Values are mean of 3 replicates ± S.D.

* Not tested since Clotrimazole is a fungicide.

Callus ethanol extract was more effective on Gram positive *S. aureus* than Gram negative *P. aeruginosa* when mixed with drugs at percentages at 25%, 50% or 75%. Administration of the extract alone was effective on Gram negative *P. aeruginosa* (Table 12).

These results exhibited that Cefotaxime sodium mixed with the callus ethanol extract produced inhibition zones larger than those resulted from undiluted treatments and callus extract only as in Appendix 2.

This may be due to the absorption of Cefotaxime sodium by skin (*in vivo*) during the treatment, and its activity had increased after addition of chamomile extract.

When Acetic acid 6% was mixed with the callus ethanol extract produced inhibition zones larger than those produced by undiluted treatments on the *S. aureus* (22.0 mm) and *C. albicans* (22.0 mm) while against *P. aeruginosa* (25.2 mm), but alone ethanol extract produced inhibition zone against *S. aureus* (35.5 mm), *P. aeruginosa* (38.7 mm) and *C. albicans* (45.0 mm) large than Acetic acid 6% alone as in Appendix 3 and Table 12.

Fusidic acid mixed with the callus ethanol extract was recorded inhibition zones lower than those produced by undiluted treatment, but alone ethanol extract produced inhibition zone against *S. aureus* (35.5 mm), *P. aeruginosa* (38.7 mm) and *C. albicans* (45.0 mm) less than Fusidic acid alone against *S. aureus* (39.7 mm) but more inhibition zone in *P. aeruginosa* (20.0 mm) and *C. albicans* (35.0 mm) as in Appendix 4 and Table 12.

The use of topical preparations (skin creams and eye ointments) containing Fusidic acid is strongly associated with the development of resistance to treatment (Mason *et al.,* 2003).

These results are in accordance with (Shah, 2003) and (Hoeger, 2004) who reported that high resistance to Fusidic acid is explained by excessive and indiscriminate use of topical Fusidic acid. Furthermore, topical Fusidic acid corticosteroid combination are extensively used for range of dermatological problems especially topic dermatitis. This together with persistent *S. aureus* colonization in patients with topic dermatitis is likely associated with rising resistant rate as pointed before.

For *C. albicans* and as indicated in Appendix 5 and Table 12. Clotrimazole mixed with callus ethanol extract (75%) gave an inhibition zone of 40.1 mm which is much larger than that of drug (Clotrimazole) only (30.1 mm).

3.9. The effect of callus ethanol extracts combined with drugs on mice skin infection *in vivo*

The callus ethanol extract of chamomile (40 mg/ml) and the drugs Cefotaxime sodium and Clotrimazole showed an obvious destroying effect of callus extract on *S. aureus* and *C. albicans* compared with drugs and controls. The infection showed swallowing, redness and filled with pus cells (Fig. 10).

Figure 10 represents 2 groups of mice, group A was infected with *S. aureus*, and group B was infected with *C. albicans*.

The first subgroup was treated with Cefotaxime sodium and chamomile callus ethanol extracts) at 50%, mice skin cured after 12 days. The second subgroup was treated with Cefotaxime sodium, mice skin cured after 16 days. The third subgroup was treated with chamomile callus ethanol extracts. Mice skin cured after 6 days. The fourth subgroup

was treated with PBS; mice were survived for 12 days then mice died because of the infection (Fig. 10).

Results showed that the best treatment was with chamomile callus ethanol extracts (Table 13).

Table 13: Effect of different treatments of chamomile callus ethanol extracts on curing of mice skin infected with *S. aureus* for the four experimental mice subgroups.

Sub grouping of experimental mice (group A)	**Types of treatment**	**Results**
First subgroup	(Cefotaxime sodium and chamomile callus ethanol extracts) at a percentage 50%	Complete cure after 12 days
Second subgroup	Cefotaxime sodium	Complete cure after 17 days
Third subgroup	Chamomile callus ethanol extracts	Complete cure after 6 days
Fourth subgroup	PBS	Died after 12 days

Fig. 10: Morphological repair of the skin infected by *S. aureus* and treated with a combination of Cefotaxime sodium + chamomile alcohol callus extract at a percentage 50%, Cefotaxime sodium, chamomile alcohol callus extract and PBS.

The group B was infected with *C. albicans*, contain four subgroups. The first subgroup was treated with Clotrimazole and chamomile callus ethanol extracts at 75%, mice skin cured after 14 days. The second subgroup was treated with Clotrimazole, mice skin cured after 25 days. The third subgroup was treated with chamomile callus ethanol extracts, mice skin cured after 5 days. The fourth subgroup was treated with PBS. Mice were survival for 8 days than died because of infection as shown in Fig. 11.

The results showed that the chamomile callus ethanol extract was the best since a complete recovery occurred within 5 days (Table 14).

Skin treated with chamomile ethanol extracts originated either from flower or callus showed better and rapid recovery than that treated with the Clotrimazole. It appears that the presence of apigenin, the most active compound of chamomile penetrates into deeper skin layers when applied topically which supports the use of chamomile as a topical anti-inflammatory agent in treating inflammations in deep tissues (Merfort and Heilman, 1994; Nmecz, 2000).

Table 14: Effect of different treatments with chamomile callus ethanol extract on curing of mice skin infected with *C. albicans* for the four experimental mice subgroups.

Sub grouping of experimental mice (group B)	**Types of treatment**	**Results**
First subgroup	(Clotrimazole and chamomile callus ethanol extracts) at 75%	Complete cure after 14 days
Second subgroup	Clotrimazole	Complete cure after 25 days
Third subgroup	Chamomile callus ethanol extracts	Complete cure after 5 days
Fourth subgroup	PBS	Died after 8 days

Group B

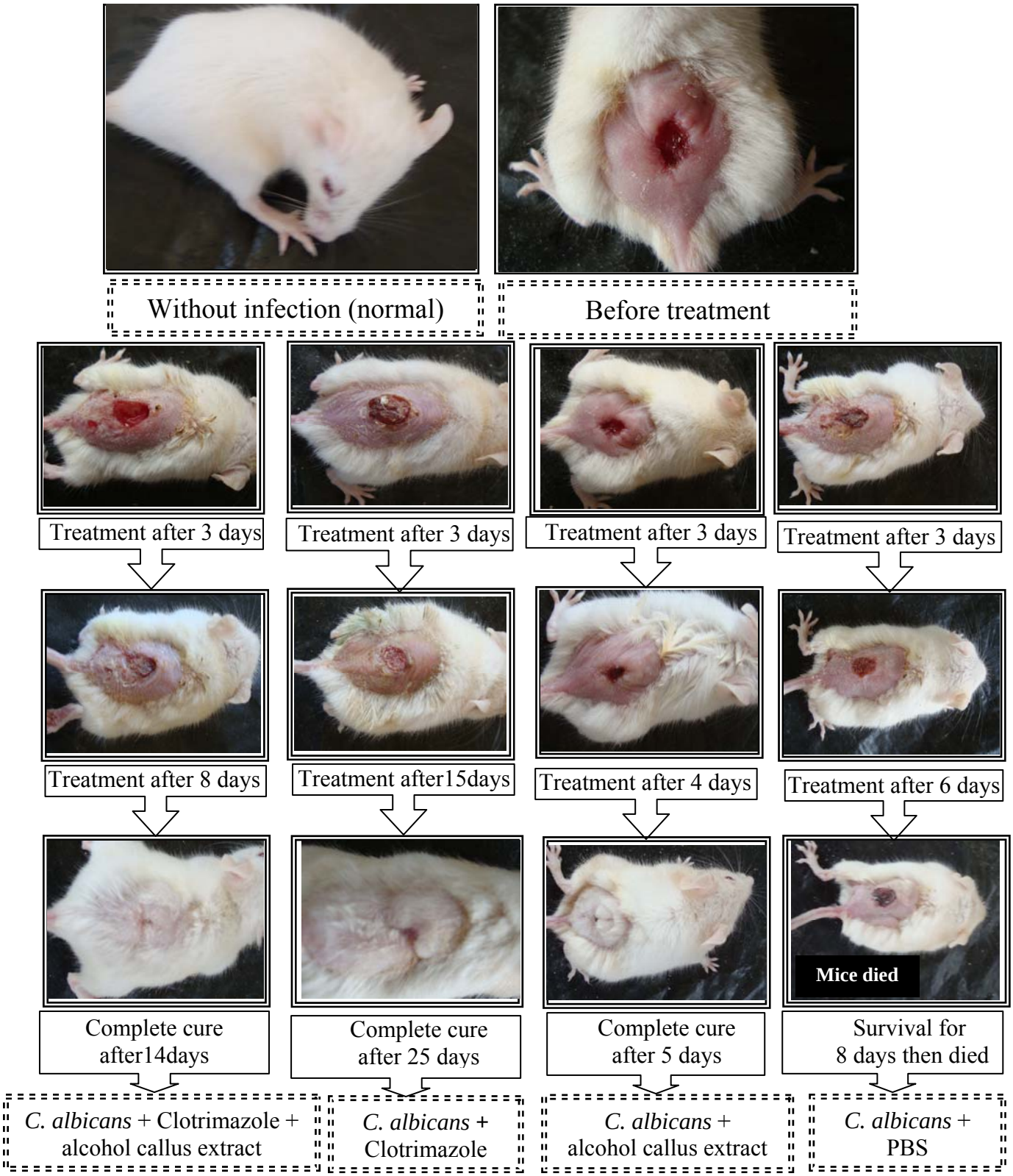

Fig. 11: Morphological repair of the skin infected by *C. albicans* and treated with a combination of Clotrimazole + chamomile alcohol callus extract at a percentage 75%, Clotrimazole, chamomile alcohol callus extract and PBS.

3.10. Phytochemical analysis using HPLC

HPLC chromatography of the used standards (apigenin and azulene) is shown in Fig. 12. Apigenin appeared at a retention time of 7.60 min. with a peak area of 1973157 mAU, while azulene appeared at a retention time of 20.72 min. with a peak area of 778417 mAU.

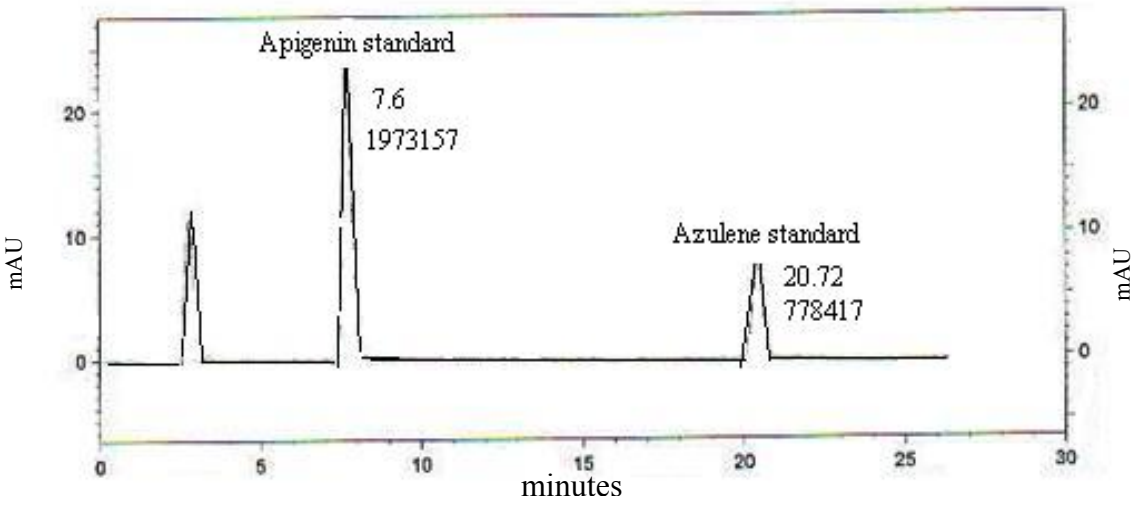

Fig. 12: HPLC analysis of apigenin and azulene standards

HPLC analysis for flower water extract showed that apigenin appeared at a retention time of 7.260 min. with a peak area of 2865286 mAU while azulene appeared at a retention time of 20.613 min. with a peak area of 102125 mAU (Fig. 13). The percentages of apigenin and azulene in water extract were 0.1162 and 0.0472%, respectively.

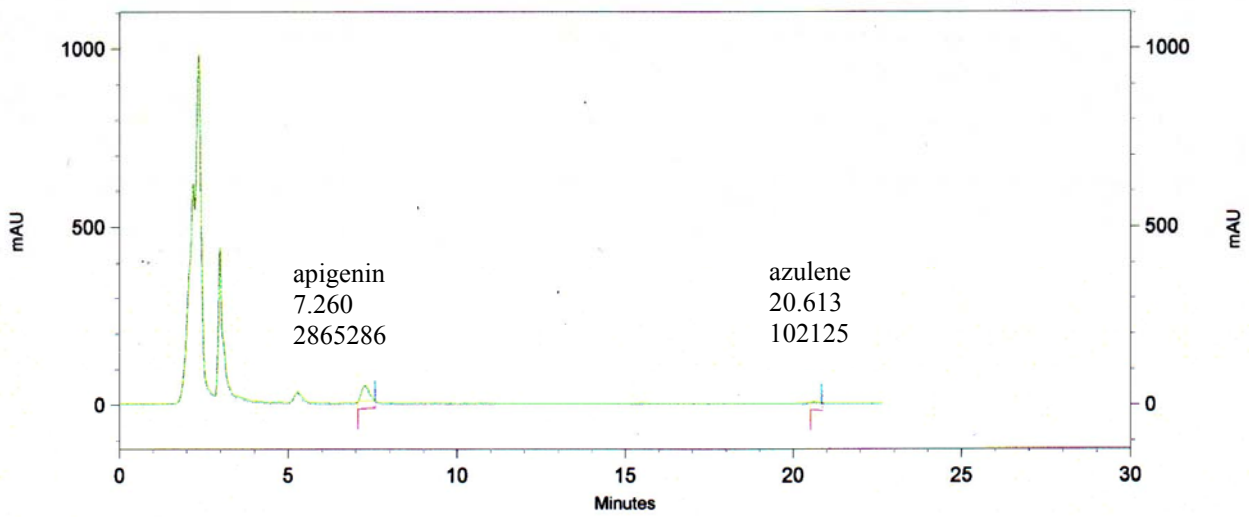

Fig. 13: HPLC of apigenin and azulene in chamomile flower water extract.

Chamomile alcohol extract of the compounds apigenin and azulene is shown in Fig 14. Apigenin appeared at a retention time of 7.211 min. with a peak area 7557442 mAU while azulene was not detected. The percentage of apigenin in alcohol extract represented 0.3064% of the crude extract.

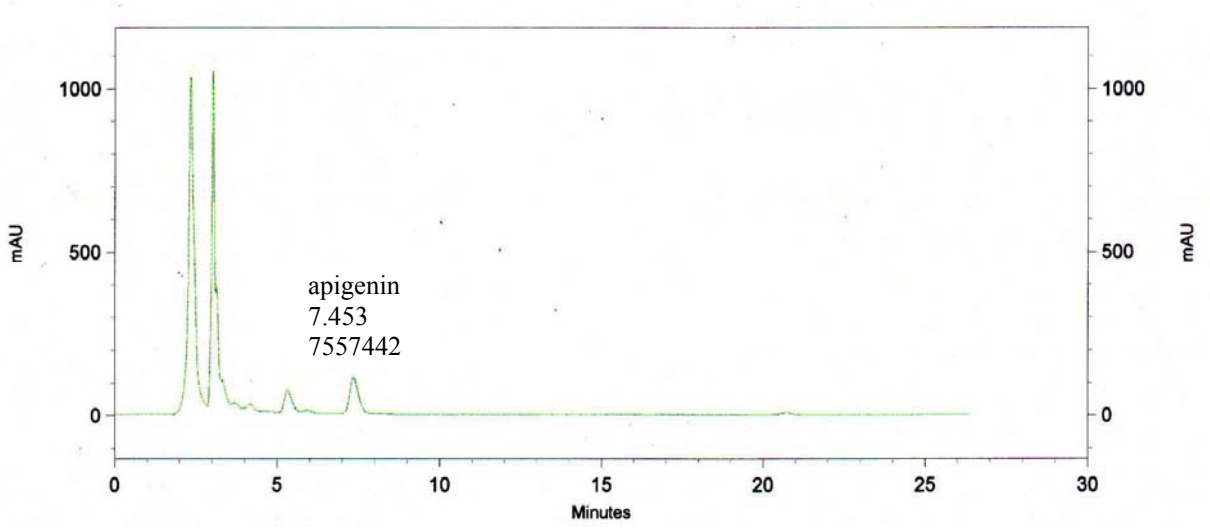

Fig. 14: HPLC of apigenin and azulene in chamomile flower ethanol extract showing appearance of the first compound and disappearance of the second.

HPLC analysis for chamomile callus alcohol extract for apigenin and azulene is shown in (Fig. 15). Apigenin appeared at a retention time of 7.453 min. with a peak area 11112284 mAU while azulene appeared at a retention time of 20.933 min. with a peak area 319260 mAU. The percentage of apigenin was 0.4505% and the percentage of azulene was 0.1476%. Apigenin and azulene were detected at 259 and 320 nm, respectively.

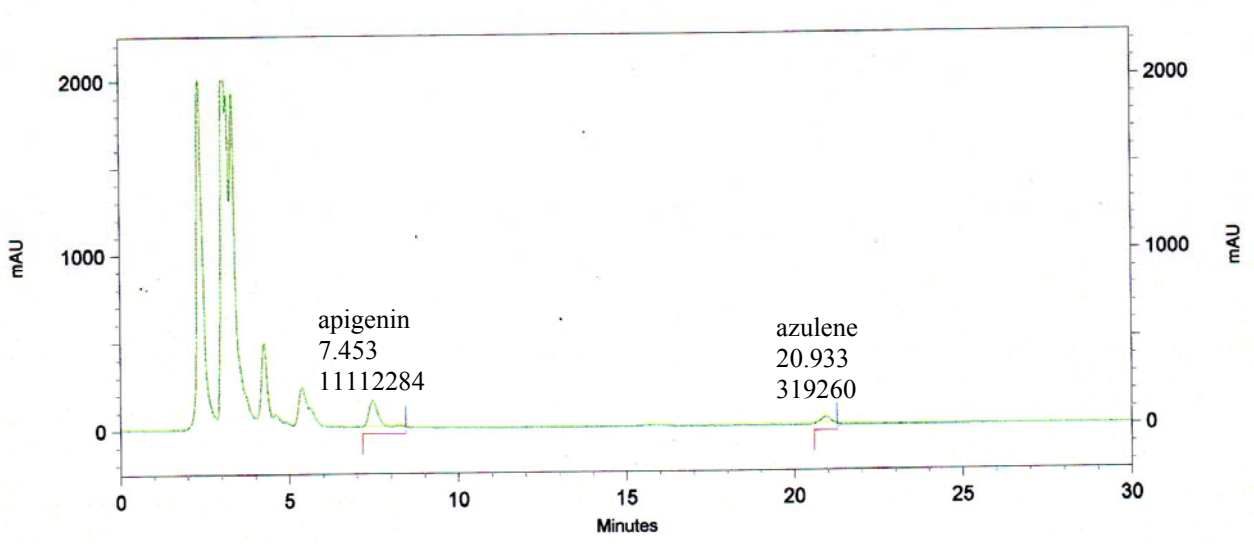

Fig. 15: HPLC of apigenin and azulene in chamomile callus ethanol extract.

From these results, apigenin was present in all chamomile extracts but azulene was present only in water and callus alcohol extracts. The highest percentage of apigenin (0.4505%) was recorded in the callus ethanol extract. This result is in accordance with Benguo *et al*., 2008 who reported that flowers of chamomile were used for apigenin extraction, which contain a percentage less than 0.3%. The pure apigenin was collected as yellow precipitate.

Conclusions and Recommendations

Conclusions

The following conclusions were obtained from this study:

1. The best concentration for chamomile seeds surface sterilization was 3% of NaOCl for 10 min. Callus cultures were induced and maintained on MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l 2,4-D using germinating seeds as a source of explants.
2. Plant tissue culture techniques are potential source for the production of some secondary metabolites.
3. Chamomile plantlets are regenerated from callus cultures grown on MS medium supplemented with 1.0 mg/l BA and 0.1 mg/l 2,4-D.
4. Flower water extracts can be used against all tested microorganisms at concentrations 40 mg/ml. The largest inhibition zone was observed against *S. aureus* and *P. aeruginosa*.
5. Callus ethanol extract showed a better inhibition activity than water or ethanol flower extracts.
6. High concentrations of chamomile ethanol flower extract (40 mg/ml) exhibited inhibitory effects against the microorganisms under investigation.
7. Ethanol callus extract is a potential cure for skin infection in tested mice.
8. The best combination of chamomile ethanol flower extract and Cefotaxime sodium at a percentage 50% against *S. aureus*.
9. The best combination of chamomile ethanol flower extract and Clotrimazol at a percentage 75% against *C. albicans*.
10. In regards to the use of antimicrobial drugs in the experimentally – induced skin infection, further studies are required to test whether these results represent a novel finding or an experimental error.

Recommendations

The following points are recommended for future studies:

1. Investigation of other medicinal plants as a source for phytochemicals using tissue culture techniques.
2. Using genetic engineering approaches for the manipulation of genes responsible for higher production of secondary metabolites.
3. Application of plant cell bioreactors for mass production of secondary metabolites.
4. Purification of the target secondary metabolite then examination for antimicrobial activity to find out which compound is the most effective.
5. Examination of extracts on other types of pathogenic bacteria and other kinds pathogens.

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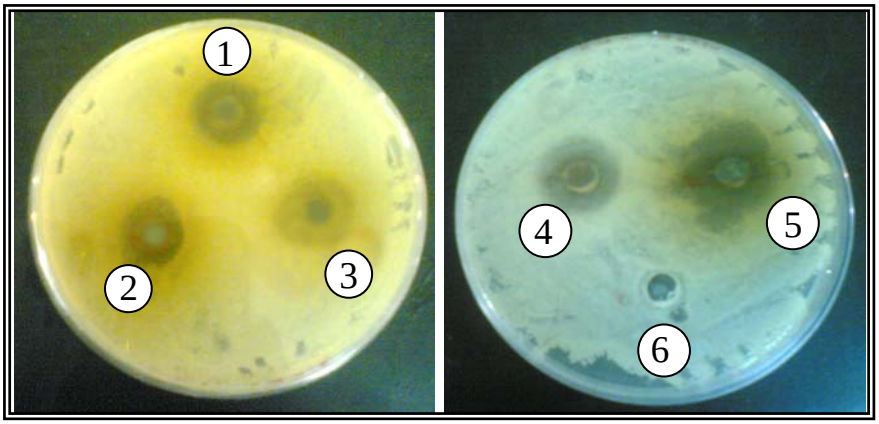
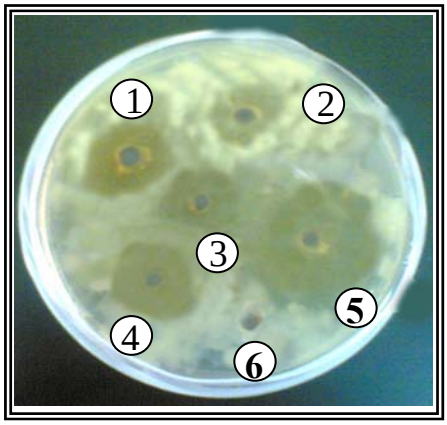
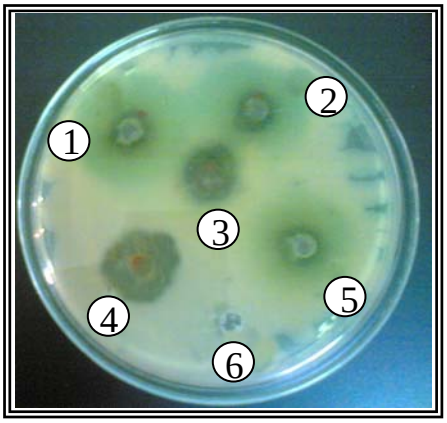
Appendices



S. aureus + Acetic acid 6%



C. albicans +
Acetic acid 6%



P. aeruginosa +
Acetic acid 6%

Appendix 1: Inhibition zones caused by treatment with Acetic acid 6% and chamomile ethanol extract

1= 25% (25% drug "Acetic acid 6%"+ 75% plant chamomile ethanol extract).

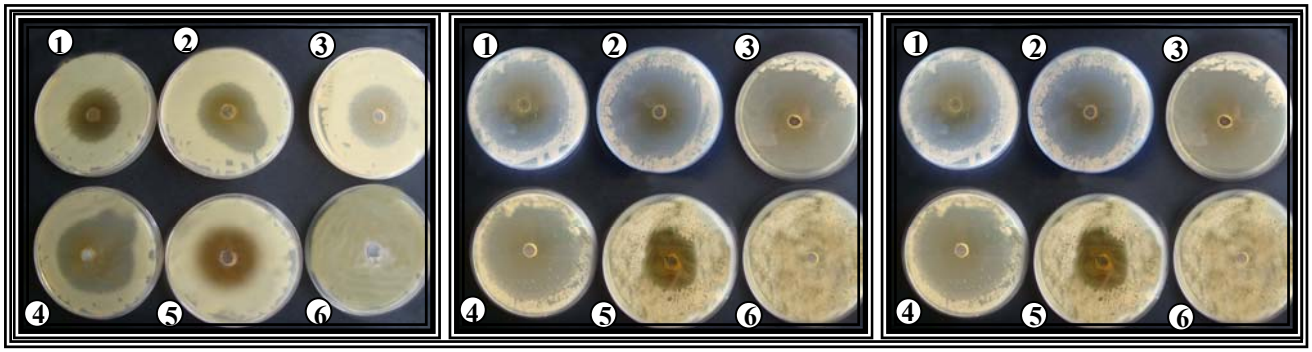
2= 50% (50% drug "Acetic acid 6%"+ 50% plant chamomile ethanol extract).

3= 75% (75% drug "Acetic acid 6%"+ 25% plant chamomile ethanol extract).

4= 100% drug "Acetic acid 6%".

5= 100% plant chamomile ethanol extract".

6= Control (PBS)



-1- *S. aureus* -2- *P. aeruginosa* -3- *C. albicans*

Cefotaxime sodium +1- *S. aureus*, 2- *P. aeruginosa*, 3- *C. albicans*

Appendix 2: Inhibition zone after the administration of Claforan and callus ethanol extracts at different ratios

1= 25% (25% drug " Cefotaxime sodium "+75% plant " callus chamomile ethanol extract).

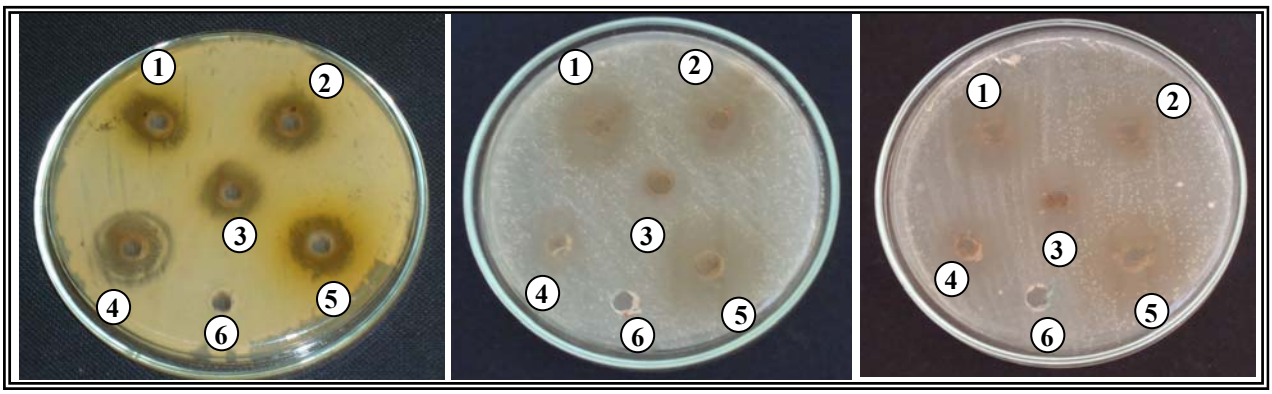
2= 50% (50% drug " Cefotaxime sodium "+50% plant " callus chamomile ethanol extract).

3= 75% (75% drug " Cefotaxime sodium "+25% plant " callus chamomile ethanol extract).

4= 100% drug " Cefotaxime sodium ".

5= 100% plant "callus chamomile ethanol extract".

6= Control (PBS).



-1- *S. aureus* -2- *P. aeruginosa* -3- *C. albicans*

Acetic acid 6% +1- *S. aureus*, 2- *P. aeruginosa*, 3- *C. albicans*

Appendix 3: Inhibition zone after the administration of Acetic acid and callus ethanol extracts at different ratios

1= 25% (25% drug " Acetic acid 6% "+75% plant " callus chamomile ethanol extract).

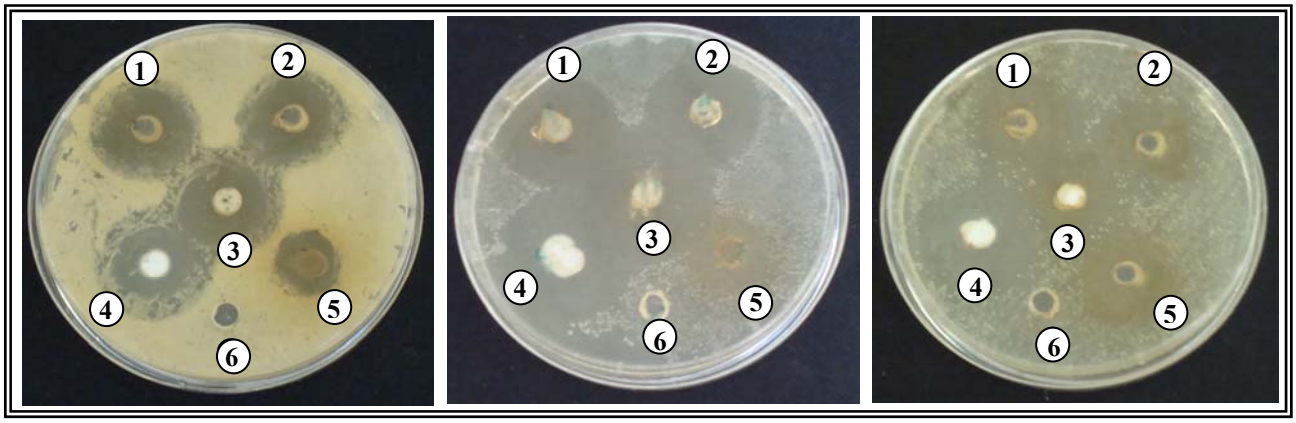
2= 50% (50% drug " Acetic acid 6% "+50% plant " callus chamomile ethanol extract).

3= 75% (75% drug " Acetic acid 6% "+25% plant " callus chamomile ethanol extract).

4= 100% drug "Acetic acid 6% ".

5= 100% plant "callus chamomile ethanol extract.

6= Control (PBS).



-1- *S. aureus* -2- *P. aeruginosa* -3- *C. albicans*

Appendix 4: Inhibition zone of Fusidic acid +1- *S. aureus*, 2- *P. aeruginosa*, 3- *C. albicans* caused by different combination Fusidic acid and callus ethanol extract

1= 25% (25% drug " Fusidic acid "+75% plant "callus chamomile ethanol extract).

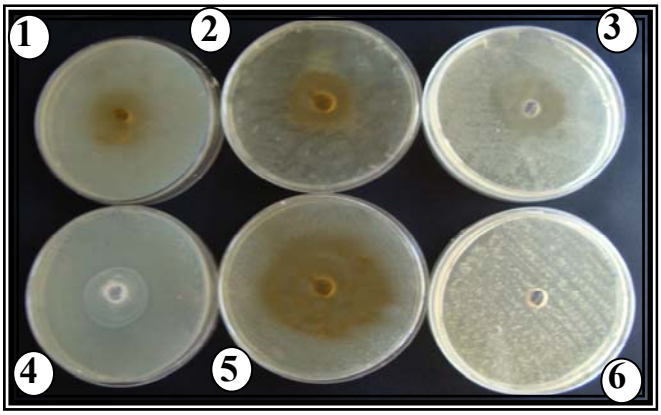
2= 50% (50% drug "Fusidic acid "+50% plant "callus chamomile ethanol extract).

3= 75% (75% drug " Fusidic acid "+25% plant "callus chamomile ethanol extract).

4= 100% drug " Fusidic acid".

5= 100% plant "callus chamomile ethanol extract".

6= Control (PBS).



Appendix 5: Inhibition zones *C. albicans* + Clotrimazol caused by different combinations of Clotrimazol and callus ethanol extract

1= 25% (25% drug " Clotrimazol "+75% plant "callus chamomile ethanol extract).

2= 50% (50% drug " Clotrimazol "+50% plant "callus chamomile ethanol extract).

3= 75% (75% drug " Clotrimazol "+25% plant "callus chamomile ethanol extract).

4= 100% drug " Clotrimazol ".

5= 100% plant "callus chamomile ethanolic extract".

6= Control (PBS).

الخلاصـــــــــــة

هدفت الدراسة الحالية إلى اختبار تأثيرالفعالية ضد الميكروبية للمستخلص المائي أو الإيثانولي لأزهار البابونج *Matricaria chamomilla* والكالس الناشيء من المزارع النسيجية للبذور النابتة وامكانية معالجة جلد الفئران المسحثة للاصابة ببعض المسببات المرضية وقورنت النتائج مع الأدوية المستعملة للامراض الجلدية مثل Cefotaxime sodium ، حامض الخليك ٦% ، Fusidic acid و.Clotrimazol اصيبت الفئران في نوعين من البكتريا هما *Staphylococcus aureus*، *Pseudomonas aeruginosa* و بالفطر *Candida albicans* والتي تسبب اصابة الجلد. استحث الكالس من بذور نبات البابونج واديم على وسط Murashige, Skoog, (MS) 1962 المجهز بـ ١,٠ ملغم/ لترمن البنزيل ادنين (BA) و ٠,١ ملغم/لتر من -2,4 داي كلورو فينوكسي استك اسد (2,4-D). أوضحتْ النَتائِجَ بأنّ للمستخلصاتِ المائية والايثانولية لزهرة البابونج تأثير مثبط لنمو الاحياء المجهرية المدروسة. كما وجد بأن المستخلصات اعلاه تحتوي على مركبات الازولين والابجينين حسب نتائج المستحصل عليها من تقنيات HPLC. كما أوضحت النَتائِجَ أيضاً بأنّ للمستخلص الأيثانولي للازهار فعالية تثبيطية ضد الاحياء المجهرية المدروسة أعلى من المستخلص المائي. وكان تأثير المستخلص الأيثانولي للكالس أعلى مِنْ المستخلصين المائي والأيثانولي للازهار. لذا فقد استعمل تركيز ٤٠ ملغم/مل من المستخلص الايثانول للكالس في معالجة الفئران المصابة وقورنت النتائج مع تأثير الادوية المستخدمة. كما اوضحتْ النتائج بأنّ التوليفيةِ الأكثر تثبيطاً للأحياء المجهرية المدروسة كانت من المستخلص الايثانولي للكالس بعد خلطها مع الكلوفيران بنسبة ٥٠% لذا استعملت هذه التوليفة في معالجة جلد الفئران المدروسة. وكان المستخلص الكحولي للكالس بتركيز ٤٠ ملغم/مل فعالاً في معالجة جلد فئران التجربة المسحثة للاصابة بالفطر *C. albicans*.

في الإعتباراتِ إلى إستعمال الادوية ضدِ المكروبيةِ في بشكل تجريبي - أقنعتْ عدوى جلدِ، دِراسات أخرى تَتطلّبُ للإختِبار سواء هناك نَتائِج تُمثّلُ إيجاد روايةِ أو خطأِ تجريبي.

الأهـــــــــــداء

للعـــطاء والحـــب والحـــنان...
لرفيـــــــقي ودربـــي...
لروحـــي مـــن جســـدي...
لمـــن أفتـــخر بهـــما وأسعـــى لكســـب رضاهـــما...
أقـــول لمـــن أعجـــز عـن الـرد القليـل ممـــاقـدما لـي...

والـــداي

إلـــى مـن كانـوا خيـر معيـن لـي....

إخـــواتي

أهـــدي لـهم ثمـــرة جهـــدي المتـــواضع.....

إينـــــاس

بِسم للهِ لرحمن لرحِيم

اللهُ نور الـسمواتِ والأرض مثَـلُ نـورِهِ كَمِشكَاةٍ فِيها مِصباح المِصباح في زجاجةٍ الزجاجةُ كَأنها كَوكَب دريُ يوقَد مِـن شجرةٍ مباركَةٍ زيتونةٍ لا شرقيةٍ ولاَ غَربيةٍ يكاد زيتها يضِئُ ولَو لَم تمسسه نارُ نـور علَى نورِ يهـدِي اللهُ لِنـورِهِ مـن يـشاءُ ويضرِب اللهُ الأمثَالَ لِلناس واللهُ بِكُلّ شيءٍ عِلِيمُ ﴿٣٥﴾

صدق لله لعظِيم

سورة النور
الآية (٣٥)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

الفعالية ضد الميكروبية لمستخلصات أزهار نبات البابونج *Matricaria chamomilla* L. والكالس في معالجة الالتهابات المستحثة تجريبياً في جلد الفئران خارج وداخل الجسم الحي

رسالة

مقدمة الى كلية العلوم – جامعة النهرين

كجزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

إينـــــاس حســـين علـي

بكلوريوس تقانة احيائية جامعة النهرين ١٩٩٩

بإشـــــــراف

كاظم محمد إبراهيم
الاستاذ الدكتور

منيرة جلوب إسماعيل
الاستاذ الدكتورة

ذو الحجة ١٤٣٠

تشرين الثاني ٢٠٠٩