Antimicrobial activity of chamomile acetone extract against some experimentally-induced skin infections in mice

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Abstract: The aim of the study was to find out the antimicrobial activity of chamomile flower acetone extract on mice skin infection and to compare the results with some traditional medicaments. Methods: A total of 2 isolates (1bacterial & 1fungi) isolated from skin lesions of infected patients. Acetonic flower extract of chamomile was investigated for their antimicrobial activity against bacterial strains, one Gram positive (*Staphylococcus aureus*), and one fungal strains (*Candida albicans*1). In this study the antibacterial activity of chamomile acetonic extracts showed highest inhibition zone (27mm) against tested bacteria isolates *Staphylococcus aureus* also it gave the high antifungal activity (18mm) against *Candida albicas*1 at concentrations 400µg/ml. Gas chromatography-mass spectrometry (GC-MS) analysis of chamomile acetone flowers extracts were detected. Vivo trials were performed on mice to determine the effectiveness of herbal plant extracts on skin. Conclusions: The study revealed that the chamomile flowers acetone extract has a higher antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*1 than the traditional drugs on experimentally-induced skin infection in mice. The study recommends further studies on other micro-organisms and on human beings.

Key words: Chamomile, Antimicrobial, Skin infections, **GC-MS**, **Topical cream**

INTRODUCTION

German Chamomile (*Matricaria recutita*) is a daisy-like flower that blooms from late spring through late summer.

It is an annual plant of the composite family Asteraceae **(Appelt, 1985**). An infusion of the flowers is taken internally as an anti-inflammatory, antiseptic, antispasmodic, carminative, diaphoretic, febrifuge, sedative, stomachic, tonic and vasodilator **(Chiej, 1984)**.

Plant use in treating diseases is as old as human civilization and traditional medicines are still a major part of regular treatments of different maladies (Alviano, 2009). Plants are no doubt one of the main sources of biologically active materials. According to a recent report medicinal herbs are used by 80% of world population living in rural areas for their primary health-care (Sakarkar and Deshmukh, 2011).

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 stimulate skin infections .Drugs used for skin infections are fucidic acid which has an *in vitro* activity against a wide range of Gram-positive and Gram-negative microorganisms. It inhibits bacterial replication and does not kill the bacteria and is therefore termed "bacteriostatic"**(Howden *et al*., 2006)**.

Nystatine was also used for treatment of susceptible fungal infections including, cutaneous candidiasis .**(Duhm *et al*., 1974).** The *in vivo* study of possible therapeutic effect of chamomile flowers acetone extracts on bacterial and fungal infections was performed on mice skin. The chamomile extracts were prepared and applied locally on the skin of experimentally infected mice described by (**Kugelberg, 2005)**.

MATERIAL AND METHODS:

Collection and extraction of plant materials

The fresh leaves of herbal plants; *Matricaria recutita L,* were purchased from the local market of Tanta, Egypt. Fresh plants (500g) were dried in the shade at room temperature then grinded into powder **(Ogunjobi and Ogunjobi, 2011).**About 20 g of dried powdered leaves were extracted with acetone (100%) concentration ,chamomile flowers were soaked for 24 h at room temperature (**Ogunjobi and Nnadozie, 2004 ; Ogunjobi *et al.*, 2007).** The resultant mixture was filtered with Whatman's No. 1 filter paper and muslin sieve to remove particles of plant sample. The clear supernatant was collected in sterile pre-weighed plate, evaporated to dryness in a rotatory evaporator at 35 ºC. The weighted crude extract was stored at 18ºC to avoid decomposition.

Collection of clinical specimens

A total of two clinical specimens were randomly collected in screw-capped container from two patients attending the outpatient clinic of the Dermatology and Venereology of Tanta University Hospitals, Egypt during May to August 2013. These include one swab from wound for bacteria and one swab from leg for fungi. For isolation of bacteria from specimens were immediately placed in 2 ml phosphate–buffered saline (PBS; NaCl, 8 g/l; KCl, 0.2 g/l; Na_2HPO_4, 1.15 g/l; KH_2PO_4, 0.2 g/l) and for isolation of fungi specimens was placed in sterile saline (0.9% Nacl) (**Dion and Kapical, 1975**; **Eman *et al*., 2009)** then transferred to Laboratory of Bacteriology and Mycology in Botany Department, Faculty of Science, Tanta University, Egypt. Each specimen for bacterial isolates was cultured on Nutrient Agar (NA). The resultant colonies in the medium were sub cultured on, Mannitol Salt Agar (MSA) for 24 h at 37 °C. The recovered isolates were subjected to different morphological and biochemical tests for the identification to the species level as described by Bergy's Manual of Systematic Bacteriology **(Kloos and Schleifer, 1975).** The isolates were identified as *Staphylococcus aureus,* identification of tested isolates was confirmed by Api–Staph and API 20E (bioMérieux) identification kit **(Palleroni, 1984)**.

For fungi each sample was cultured on Sabrouaud's dextrose agar (SDA) with adding 0.5mg/ml cyclaheximide and 0.4 g/ml chloramphincol as antibacterial agent **(Dion and Kapical, 1975; Eman *et al*., 2009).** SDA were incubated for 2 days at 37 °C for yeast species. The obtained colonies were examined and identified microscopically under light microscope according to (**Moubasher 1993; Clyton and Midgley1985).** The

resultant yeast colonies were biochemically identified as *Candida albicans* 1, using API 20 C AUX (bioMérieux) identification kit **(Buesching *et al*., 1979; Ahmed *et al*., 2008**).

Gas chromatography Mass Spectroscopy for chamomile flower extract:

Gas chromatography-mass spectrometry (GC-MS) analysis was used to determine chemical composition of acetonic chamomile extracts by GC-MS by gas chromatography, Masse Spectroscopy in Claurs 580/560S. Work was done with column 30.0m x 250µm, Rtx5MS (crossbond 5% diphenyl 95% dimethylpolysiloxane), Perkin Elmer Company in Central lab, Tanta University, equipped with heated FID, Egypt.

The antimicrobial activity of the extract was determined against target microorganisms (*S. aureus*, and *C. albicans*1) *in vitro* by using modified agar diffusion method describe by **(Nathan, 1978)**.The extract was prepared at four concentrations100, 200, 300 and 400µg/ml to determine the antimicrobial activity for each concentration. The dried acetonic extract was redissolved with acetone (100%) concentration.

Five evenly spaced wells, 6mm 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 (50µl) acetone.

Equal volumes of the four concentrations 100, 200, 300or 400 µg /ml of the extracts were dispensed into each well (three replica plates were prepared for each agent). Test plates were then incubated at 37°C for 24 hrs for bacteria and yeast isolates, the zones of inhibition were measured using a ruler. A clear zone indicated that the extract showed its antibacterial or antifungal activity. This method was repeated three times for each test. (The values were averaged for the three experiments).

Preparation for cream:

Topical creams were prepared from the chamomile flower acetone extract .Preparation of topical creams was carried out according to **(Purushothamrao *et al.*, 2010)** at the Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, Tanta University. Formulation of 50 g containing 0.5% of active ingredients was com-posed of two phases (oil phase: cetostearyl alcohol, 4.0 g; vaseline 7.5g; liquid paraffin 3.75 ml; and aqueous phase: deionized water, 35 ml; sodium dodecyl sulphate (SDS), 425 mg; active ingredient 250 mg). Ingredients of oil phase were mixed together by melting in China dish on constant stirring. Components of aqueous phase were mixed together and warmed to about same temperature of oil phase. Aqueous phase was added to oil phase drop by drop on constant stirring until solidification. The preservative propyl paraben and methyl paraben were added after cooling**.**

Experiment Treatment

Mice were divided into two groups: group A (infected with *S. aureus*) and group B (infected with *C. albicans1*). Then each group is subdivided into four subgroups (n=3) as follows: (G1): negative control, not injected not treated (G2): treated with placebo cream,

(G3): Treated with topical chamomile flower extract cream (G4): Positive control, treated with fucidic acid or nystatine for each group.

For bacterial infection, the back hair of all mice were shaved and their skin was sterilized with iodine and wiped sterile water .The sublethal dose premeasured ($2x10^7$ CFU /mouse) of the selected *S.aureus* isolate was injected subcutaneously into group G2– G4 **(Leedy, 1997).**

For fungal infection, the back hairs of all mice were shaved and their skin was sterilized. Mice were swapped with the selected fungal isolates (((10^6CFU/ml) of *C. albicans* 1 on the center of the mice's back **(Back *et al*., 1985**). Followed by adding of 0.1 ml olive oil, the infected area was covered with plastic film using leucoplast tap, after 3 days of skin infection, tested creams were loaded separately on the infected lesion twice daily. All treated and control group were observed for 17days. Inspecting of the healing lesions, the repairing to the normal skin are observed through the topical treatment of wounds and the growing hairs upon the repaired skin were recorded.

RESULTS:

Chamomile flowers acetone extracts exhibited antibacterial and antifungal activity against *staphylococcus aureus* and *candida albicans*1. The antibacterial activity was shown at different concentration (100,200,300 and 400 µg /ml). (table1)

At regards *Candida albicans1* chamomile flowers acetone extracts showed no inhibition zones at concentration 100 and 200µg /ml and gave14mm at concentration 300 µg /ml.

Table 1: Mean diameter of inhibition zones caused by chamomile flower acetone extract at various concentrations on *Staphylococcus aureus* and *Candida albicans*1.

Isolates	Mean of inhibition Zone (mm)			
	Concentration of acetonic extract (µg/ml)			
	100	**200**	**300**	**400**
Control	0± 0.00	0± 0.00	0± 0.00	0± 0.00
Staphylococcus aureus	15± 0.18	21± 0.8	25± 0.06	27± 0.145
*Candida albicans*1	0± 0.00	0± 0.00	14± 0.02	18± 0.22

Control = Acetone solvent, control =0, 0 = No zone of inhibition, no antimicrobial activity
Values are mean of three replicates ± S.D.

Table (2) show four subgroups of mice (each group contain three mice) were subcutaneously injected with sublethal dose ($2x10^7$ CFU/ml/mouse) of *Staphylococcus aureus.* First group of mice let it healthy to make comparison, second group was treated with placebo cream, third group was treated with chamomile flower extract cream on the wounded area and the fourth was treated with fucidic acid cream. The diameter of wounded area of each treated mice was measured every three days after sprayed with each tested treatment (chamomile flower extract, fucidic and placebo) until recovery. There was decrease in the wound area throughout the experimental period in all groups. However, the reduction in the wound area in the placebo treated group was less dramatic when compared to the flower of chamomile plant extract and fucidic treated groups. After one week, the treated lesions with chamomile flower extract cream were reduced effectively and the treatment was very effective after two weeks illustrating complete

lesion disappearance without any inflammation as in photo (D) while in case of fucidic cream treatment small lesion were observed with inflammation as in photo (C), while in photo (B) the treatment with placebo large lesion appear with inflammation and redness.

Table 2: Effect of different treatments on *S. aureus* skin infection of mice:

Mice group A	Days	Treatment	Result
Group (G1)	1st day	Not Injected	Healthy mice
	2nd - 17th days	No topical application	
Group(G2)	1st day	Injected with 10^7 CFU/ml of tested bacteria	Still redness and inflammation on the skin after 17 days
	2nd - 17th days	Topical application of formulated cream placebo (twice daily)	
Group(G3)	1st day	Injected with 10^7 CFU/ml of tested bacteria	
	2nd - 17th days	Topical application of formulated cream (twice daily)	Complete cure after 14 days
Group (G4)	1st day	Injected with 10^7 CFU/ml	
	2nd - 17th days	Topical application of antibiotic fucidic acid cream (twice daily)	Less redness and inflammation

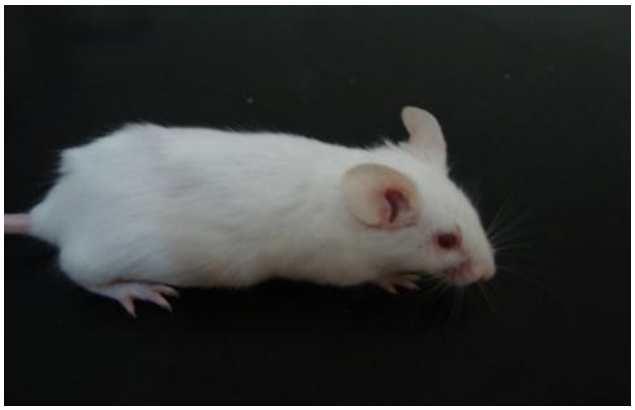

Fig (1): Induced healthy mice as Control (without infection)

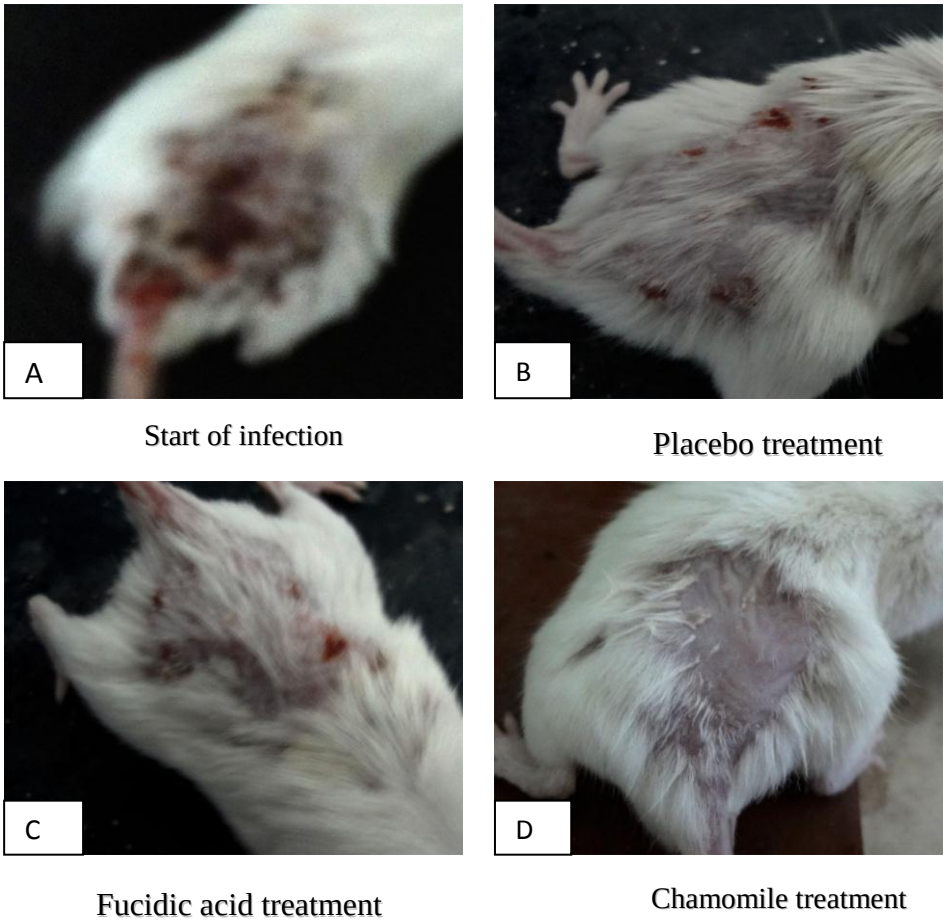

Fig (2): Treatment of the skin infected by *Staphylococcus aureus* **Fig A.**Induced wound of mice before treatment process. **Fig B.** Induced wound of mice after treated with placebo **Fig C**. Induced wound of mice after treatment with fucidic **Fig D**. Induced wound of mice after treatment with chamomile flower extract cream.

Table (3) Show four subgroups of mice(each group contain three mice) the first one was for healthy mice's skin tissues the second was for infected mice 's skin tissues with *Candida albicans*1 and treated with placebo ,the third was for treated mice's skin tissues with chamomile plant extract cream and the last one was for treated mice's skin with nystatine cream .

After one week, the treated lesions with plant cream were reduced effectively and the treatment was very effective after two weeks illustrating complete lesion disappearance without any inflammation and the lesion covered with hairs as in photo (D) while in case of nystatine treatments small lesion were observed with inflammation as in photo(C) while in photo (B) the treatment with placebo large lesion appear with inflammation and redness.

Table 3: Effect of different treatments *C. albicans1* skin infection of mice

Mice group B	Days	Treatment	Result
Group(G1)	1st day	Not infected	Healthy mice
	2nd - 17th days	No topical application	
Group(G2)	1st -3rd day	Infected with 10^6 CFU/ml of tested fungal isolates	Still redness and inflammation on the skin after 17 days
	4th - 17th days	Topical application of formulated cream placebo (twice daily)	
Group(G3)	1st - 3rd day	Infected with 10^6 CFU/ml of tested fungal isolates	Complete cure after 14 days
	4th - 17th days	Topical application chamomile plant extract cream (twice daily)	
Group (G4)	1st - 3rd day	Infected with 10^6 CFU/ml	Less redness and inflammation
	4th - 17th days	Topical application of anti fungal cream Nystatine (twice daily)	

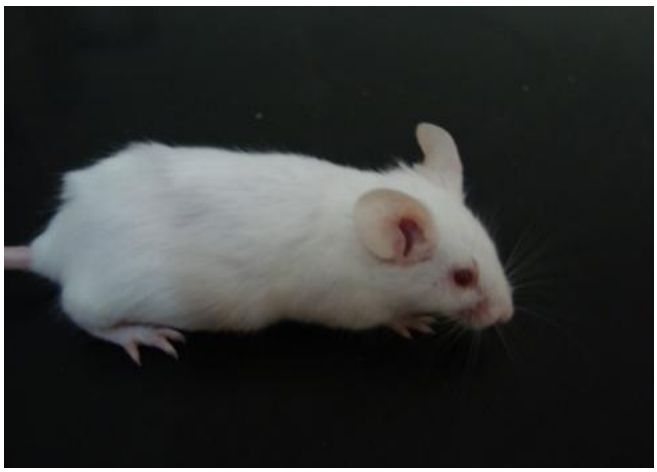

Fig (3): Induced healthy mice as Control (without infection)

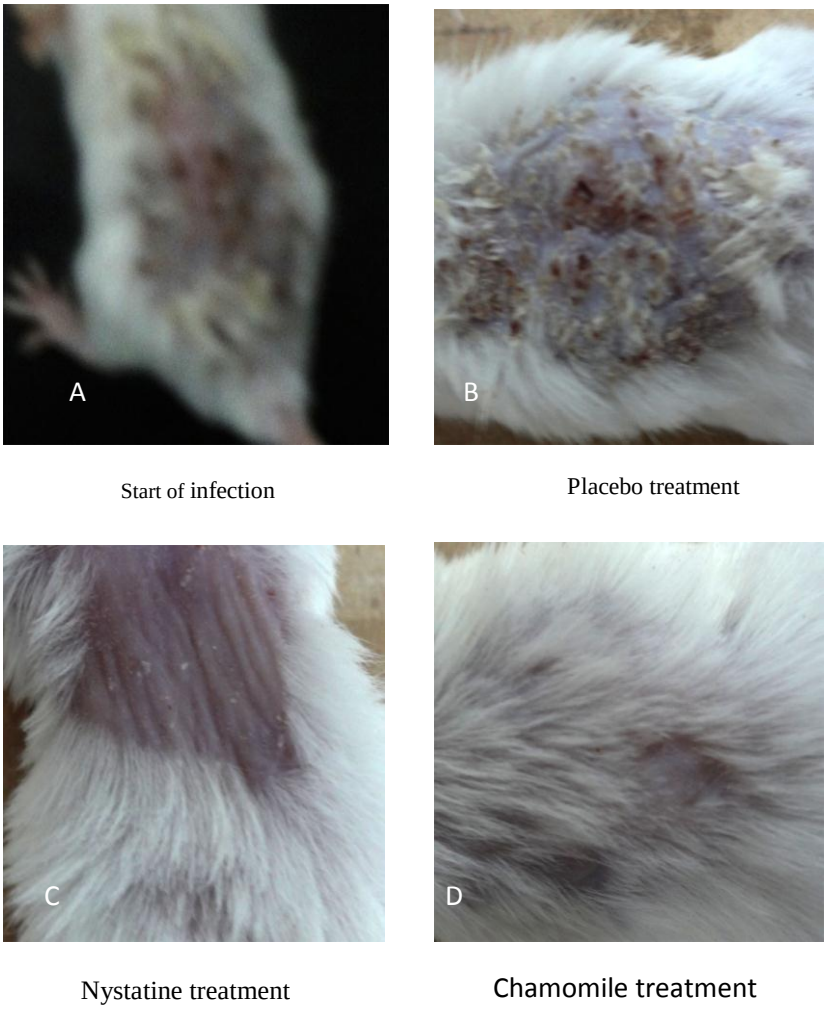

Fig (4): Treatment of the skin infected by *C. albicans1* **Fig A.** Induced wound of mice before treatment process. **Fig B.** Induced wound of mice after Treatment with placebo **Fig C**. Induced wound of mice after Treatment with nystatine **Fig D**. Induced wound of mice after Treatment with chamomile plant extract cream.

GC-MS for Chamomile flowers acetone extracts:

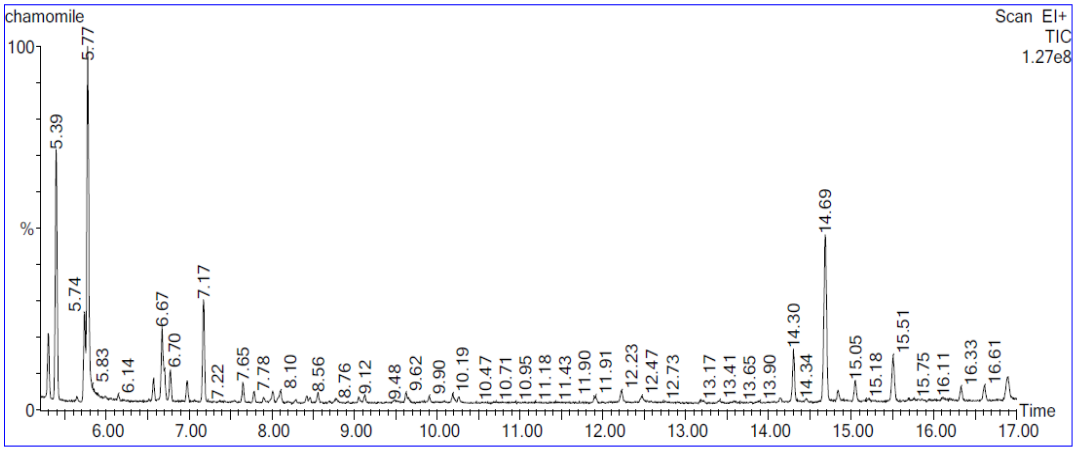

Fig (5): GC-MS chromatogram of chamomile flower acetone extracts

Table 4: GC-MS analysis of different compounds of chamomile acetone extracts

Peak	Rt (min)	Area%	Name of compound
1	5.298	2.857	Ethylbenzene, m-Xylene, p-Xylene
2	5.39	11.408	m-Xylene , Benzene, 1,3-dimethyl-
3	5.739	3.956	o-Xylene , Benzene, 1,3-dimethyl-
4	5.774	19.29	Ethanol, 2-butoxy.
5	6.6 74	4.526	Benzene, 1-ethyl-2-methyl-
6	6 .77	1.37	Benzene, 1-ethyl-3-methyl
7	7.17	4.81	Benzene, 1,3,5-trimethyl-
8	14.30	2.997	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene
9	14.687	11.076	Anthracene, 1,2,3,4,5,6,7,8-octahydro-1-methyl
10	15.052	1.217	1-Phenyl-1-nonyne ,
11	15.51	3.090	Palmitic acid , Pentadecanoic acid, Hexadecanoic acid,

Rt, Retention time

The GC-MS chromatograms show many compound present in chamomile acetone extract. Name of these compound and their amounts are listed in table (4).

In the chamomile acetone extract the major component o-Xylene , m-Xylene , Benzene, 1,3-dimethyl(15.3 %) which have antimicrobial and antifungal activity .The extract also contain Ethanol, butoxy (19.29%) which have 2-alkoxy derivative for

antifungal agent so it has anti fungal activity, the extract also contain many antimicrobial fatty acid such as palmitic acid , pentadecanoic acid, hexadecanoic acid (3.0905%), benzene, 1-ethyl-3-methyl(5.5%)1-phenyl-1-nonyne (1.217).Most of components were alkaloids , terpenoids and phenolic compound .

DISCUSSION:

In the present study, the inhibitory effect of chamomile flower acetone extract on *Staphylococcus aureus* and *Candida albicans* were in agreement with **(AL-naymi , 2005)** who reported that acetone extracts of chamomile flowers have higher activity against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus pneumoniae, Micrococcuse ssp.*and *C. albicans.*

Crotteau *et al.*, 2006 showed antimicrobial activity of chamomile against *candida albicans* also (**Ghaly *et al.*, 2010)** illustrated that chamomile showed antimicrobial activity against *B. subtilis* and *C.albicans* that may be due to the nutrients in these herbs.

Discussing the results of GC-MS analysis of the chamomile extract conclude that the potential antimicrobial activity is attributed to different compounds belonging to a diverse range of chemical classes **(Ozdemir *et al.*, 2004).** These compound could be linked to synergic effects, leading to disruption of the cellular membrane of fungi and bacteria because they can penetrate the extensive meshwork of peptideglycan in the cell wall without visible changes and reach the bacterial or fungal membrane leading to its disintegration (**Benkendorff *et al.*, 2005 ; Bergsson, 2005; Mendiola *et al.*, 2007; Kumar *et al.*, 2011).**

Santhamari *et al.*, 2011 illustrated that antimicrobial activity of chamomile acetone extract which had inhibitory activity against gram negative and gram positive bacteria is due to the presence of active ingredients that inhibit bacterial and fungal growth. Antimicrobial activi1ty may be due to numerous free hydroxyl ions that have the capability to combine with the carbohydrates and proteins in the bacterial and fungal cell wall they may get attached to enzyme sites rendering them inactive.

In present study the artificial wound were healed (100%) after 14 days in case of chamomile extracts cream and after 17 days with nystatine cream and fucidic cream (**Chah *et al.*, 2006**) It appears that the presence of the most active compounds of chamomile, penetrates into deeper skin layers when applied topically which supports the use of chamomile as atopical anti-inflammatory and antimicrobial agent **(Merfort *et al.*, 1994). Conclusion** The study revealed that the chamomile flowers acetone extract has a higher antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*1 than the traditional drugs on experimentally-induced skin infection in mice. GC/MS analysis found that plants have active compounds like fatty acid; ester, alkaloids, hydroxyl group and phenolic that might give these plants the antimicrobial activity**.**

ACKNOWLEDGMENT:

Authors thank Dr. Shereen F.Gheida, Department of Dermatology and Venereology, Faculty of Medicine, Tanta University, Egypt, Who have helped in collecting samples from patient.

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