Effect of Flower Extracts of Matricaria chamomilla L. on Some Bacteria Causing Eye Infections.

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Abstract

Water and alcoholic extracts *of Matricaria chamomilla* flowers were investigated for their antimicrobial activity(with concentrations 5,10,20,40 mg/ml) .Results of chamomile flowers water extract showed better inhibitory effect than ethanolic one against: *Staphylococcus aureus and Bacillus cereus* and to alesset to *Pseudomonas aeruginosa* and *Escherichia coli*.It has been noticed that all extracts that anti-inflammatory effectat concentraion of 40mg/ml . However,these extracts showed antibacterial activity at acgncentration of 40 mg/ml that applied on laboratory rabbit eyes.

Introduction

Medicinal plants are gifts of nature, against various infecations and diseases.In many parts of the world, herbs were used as food(vegetables) and flavors for centuries.Some herbals plants are considered as house medicine and played important role in nearly most cultures and all over the contents(1). Chamomile (*Matricaria chamomilla)*,is one of the widly used and well-documented medicinal plant in the world.It is included in the the pharmacopoeia of 26 counteries(2,3).Chamomile prefers sandy soil and full sun.They grow in late spring or early summer .Flowers are harvested throughout summer when they are fully open(4,5).German chamomile and Roman chamomile are the two major types of chamomile.These two types have similar medicinal properties. The flower is the main medicinal part of the herb. Chamomile products can be administrated in many ways. The common ones are:Oral,Inhalation, and Solution for bath and infusion(6). German chamomile may be slightly stronger.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 suchas *:Staphylococcus aureus, S.aureus , S.epidermidis Streptococcus pyogenes, Strepticoccus pnemoniae, Micrococcus spp and. pseudomonas aeruginosa* . Both types of chamomile contain minute amounts of blue oil (azulene) .This oil has neutralizing abilities on the toxins produsedby various bacteria and therefore, assists in the healing processof wounds(5).The importance of the secondary metabolites in plants are:to protect the plants against being eaten by herbivores and against being infected by microbial pathogens.They serve as attractants for pollinators and as agents for plant competition.Plant secondary metabolites can be divided into three chemically distinct groups:Phenolics, Terpenes and Nitrogen - containing compounds(7).The essential oil of chamomile flowers is water in soluble contains compounds responsible for many uses(8).Chamomile flowers are used internally and externally to treat extensive list of conditions.Chamomile is used as askin wash or compress,it is use on skin to increase wound healing and reduce inflammations such as those caused by allergies and pathogenic microorganism.It is used for treatment of teething pains and eye infections (9,10).Although chamomile is widely used, there is not enough reliable research in humans to support, it is use for many conditions.So this study amid to preparation of extracts(water and ethanolic)

from chamomile flowers and examination of these extracts for their antibacterial activity against some patho-genic bacteria causing eye infections.

Materials and methods

Plant material

Chamomile*(M.chamomilla* L)dried flowers were bought from local market.

Bacterial isolates

Four bacterial isolates were obtained from patients with eye infections(Ibn AL-Haetham Hospital) included: *S.aureus,B.ceries,E.coli and Ps.aeruginsa.*All isolates were identified by biochemical testes.(11)

Experimental animals

Nine local rabbits of either sex,(5-6) months of age and weighting approximately (1.5-2)kgm were used in the study.

Water extract method.

Plant powdered material was macerated with DDH_2O inaratio 1:5(w/v),50gm of the powder was mixed with 250ml DDH_2O .The mixture macerated over night at room teamperature(25◦C).The suspension was filtered through out filter of gauze to getride of the large particles then filtered through afilter paper (watman no.1). The filtrate was concentrated using arotary evaporator at 40◦C(12).

Ethanolic extract method.

Aquantity of 50gm of plant powder was extracted with 250ml of 75% ethanol by sowhlet apparatus for 6thr.at(40-60) c˚,thene the solvent was removed under reduced pressure by rotary evaporator at 40c. The crude solid extract was kept in deep freeze until use(13).

Determination of the antibacterial activity of extracts(in vitro).

The activity of extracts were determined against (bacterial isolates) *in vitro* by using modified agar diffusion method.For two extracts(water and ethanolic),the solutions were prepared by dissolving 5gm of each flower extracts residue with 50ml sterile DDH2O.The extracts were prepared at different concentrations(5,10,20 and 40)mg/ml.The medium(Brain – Heart infusion agar)was poured in Petri-dishes,and inoculated with 0.1ml of(1.5x10 CFU/ml) of isolates (*S.aureus, B.cereus,E.coli and Ps. aeruginosa)*by using sterile swabs.Five evenly spaced holes 3mm indiameter were made in the agar of each plate with sterile corkborer.To identifiy the intrinsic extracts activity(water and ethanolic),one control well was filled with 100µl phosphate buffer saline (PBS). Anequal volumes of different concentrations (5,10,20and40)mg/ml of the extracts-were dispensed into each well (Four replica plates were prepared for each concentration).Test plates were there incubated at 37c˚ for 24hr.and zones of inhibition were measured using aruler in millimeters .Aclear area indicated that the extract showed its antibacterial activity.This method was repeated twice for each extract(14).

The effect of chamomile extracts on rabbit eyes(*in vivo*).

Aliquot of 0.5ml of(40mg/ml) extracts (water and ethanolic) ofchamomile were mixed with 0.5ml of nutrient broth medium, then 0.1ml of ($1.5x10^2$CFU/ ml) bacterialsuspension*(S.aureus,B.cereus,E.coli* and *Ps.aeruginosa)* were inoculated As acontrol ,amixture was prepared individually, containing 0.5ml of nutrient brot medium ,0.5ml of PBS and 0.1ml of bacterial suspension. Samples and control were incubated at 37c˚ for 24hrs.Nine local rabbits were administrated with an intrastromal injedtion of samples (0.1ml/left eyes).Right eyes for the some rabbits were injected with control sample. Results were recoreded 24hrs.after injection(15).

Statistical analysis:

Acompletely randomized design (CRD) was used ,Least significant differences (LSD) were calculated.Means compared at probability of 0.05(16).

Results and discussion

Effect of water and ethanolic extracts on the growth of some bacteria(*in vitro*).

Results of the effect of water extract displayed in table(1) indicate the high concentrations of extract(20 and 40)mg/ml has inhibitory effects against Gram-positive bacteria(*S.aureus*) with (16.3 and 18.2)mm inhibition zones diameter respectively, while 13.6mm was recorded in the concentration 10mg/ml.Slight inhibition was observed at the concentration of 5mg/ml(Figure 1-a) while *B.cereus* gave 13.5mm in aconcentration of 20mg/ml,18mm in aconcentration of 40mg/ml,and the concentration 10mg/ml showed 13.0mm and slight inhibition was observed at concentration of 5mg/ml(Figure 1-b).The inhibitory ability was mor pronounced against *S.aureus*,whereas it showed less activity against Gramm-ve bacteria*(E.coli* and *Ps.aeruginosa*).In *E.coli*, the high concentrations of water extract(20 and 40)mg/ml showed(15 and16)mm inhibition zones subsequently and 13.0 mm in the concentration 10mg/ml.Slight inhibition was observed at the concentration of 5mg/ml(Figure 1-c) ,while *Ps.aeruginosa* showed aslight inhibition at 5mg/ml. The concentration 10mg/ml showed 13.3mm inhibition zone,the inhibition zones at(20 and40) mg/ml were(15.4 and 16.3) mm of extract subsequently(Figure1-d).Results agree with (17) who regarded Gram-positive bacteria specially *B.ceries* and *S.aureus* are sensitive to *M.chamomilla* water extract,Gram-negative bacteria (*E.coli* and *Ps.aeruginosa*) were relatively less sensitive.The antibacterial activity may depend on the concent-ration of chamazulen, bisbolol and bisabolol oxides(A and B) in the extract. Even at concentrations ,lower than 100µ/ml (bisbolol and its spiro-ethert were effective antibacterial agents)(10).The resistance of Gram-negative bacteria could be dueto the permeability barrier provided by cell wall(18).The results are also in agreement with (19) who showed the role of chamomile in preventing infections bacteria suchas *S.aureus*.

Effect of ethanolic extract.

Chamomile ethanolic extract exhibited antibacterial activity against micro-organism at the concentrations (20 and 40)mg/ml(Table2).The diameter of the inhibition zones against *S.aureus* was 15.3mmat 40mg/ml.Whereas,decreas to 12.6mm at the concentration 20mg/ml. The lower concentration of extracts (10and5)mg/ml showed 10.8mm and slight inhibition

respectively for S.aureus(Figure2-a)..*B.cereus* was inhibited at extract concentrations(10,20 and 40)mg/ml.It showed 12.3,14.3 and 16.4mm inhibition zones diameter(Fig-ure2-b). *E.coli* showed (11 and 14.2)mm at(20 and 40)mg/ml respectively.Slight inhibition was observed at the concentration 10mg/ml(figure2-c). Whil *Ps.aeruginosa,*showed slight inhibition at10mg/ml and inhibition zones at(20 and 40)mg/ml subseguently were (12.0 and 14.0)mm respectively (Figure 2-d).It appears that wayer extract is more efficient that ethanolic .The reason may be due to the the compounds already extracted by water particulary flavan-oids,This results disagree with (19) who reported that ethanolic extracts of *M.chamomilla* flowers have higher activity than water one.It is clear from the data presented in tables 1 and 2 that among the four tested microorganisms, *S.aureus* was the most susceptible microb to the extracts. Furthermore, our results are in agreement with(18), who showed that chamomile extracts(water and ethanolic) are widely used as anti-inflammatory and antibacterial activity.Although *M.chamomilla* contains many active compounds,most studies arttitu-bied,the antimicrobial activity in chamo-mail to terpene ompounds(21,22). (20) suggestedthat the activityof M.*chamomilla* could be attributed to the existenschamazulen,abisabolo(sesquiterpene) that showed high inhibition activity Sagainst*S.aureus,S.epidermidis,St.pyogenes, Micrococcus* spp. and *Candida albicans* .

The effect of M.chamomilla extracts on rabbit eyes(in vivo).

The extracts of chamomile showed on obvious effect on destroying *S.aureus* cells in the rabbit eyes after injection at aconcentration 40mg/ml (Figure-3) compared with controls,the control showed swallowing closed eyes and filled with pus cells(Figure-4).Water extract of chamomile showed abetter effect than ethanolic one on damaging *S.aureus* cells in the rabbit eyes.Our results agree with(10),who showed that chamomile tea help to relieve eyes redness and swollen eyes.(9) coducted *in vivo* study on nine female volunteers. They stared that chamomile absorbed at the skin surface and penetrared into deeper skin layers.This observation supports their use as topical antiphlo-gistic agents to treat inflammations in deep tissues suchas cornea.

Table (1): Diameter of inhibition zones caused by *M. chamomilla* flowers water extracts at various concentrations on some G+ve and G-ve bacteria.

Conc. Mg/ml dwt	Diameter of inhibition zone (mm) of bacterial isolates ± S.D.			
	S. aureus	*E.coli*	*B. ceries*	*P. aeruginosa*
40	18.2±0.75	16.0±0.96	18.0±0.20	16.3±0.84
20	16.3±0.24	15.0±0.65	13.5±0.62	15.4±0.44
10	13.6±0.47	13.0±0.70	13.0±0.70	13.3±0.79
5	Slight inhibition	Slight inhibition	Slight inhibition	Slight inhibition
Control (PBS)				

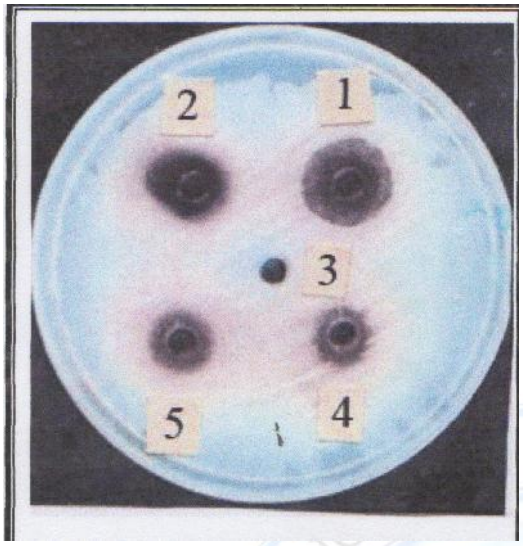
Values = are mean of 3 replicates ±S.D.

Table (2): Diameter of inhibition zones caused by *M. chamomilla* flowers ethanolic extracts at various concentrations on some G+ve and G-ve bacteria.

Conc. Mg/ml dwt	Diameter of inhibition zone (mm) of bacterial isolates ± S.D.			
	S. aureus	*E.coli*	*B. ceries*	*P. aeruginosa*
40	15.3±0.62	14.2±1.4	16.4±0.82	14.0±0.71
20	12.6±0.61	11.0±0.81	14.3±0.49	12.0±0.94
10	10.8±0.23	Slight inhibition	12.3±0.62	Slight inhibition
5	Slight inhibition	-ve	Slight inhibition	-ve
Control (PBS)				

-ve = no activity was observed.

Values = are mean of 3 replicates± S.D.



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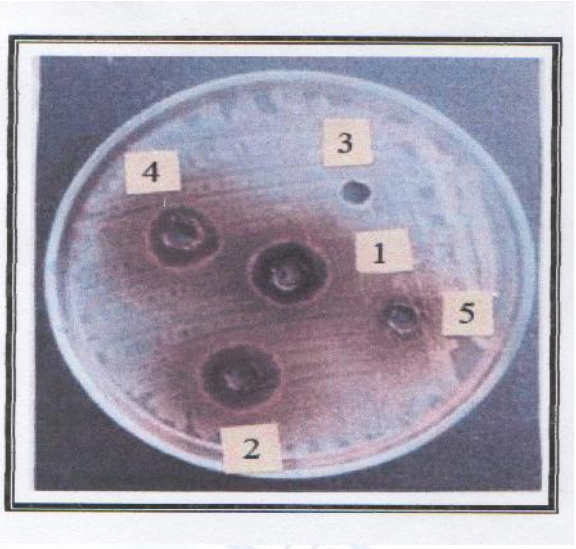

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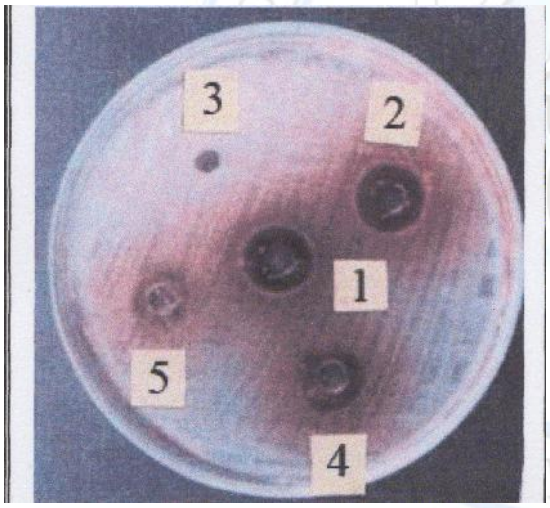

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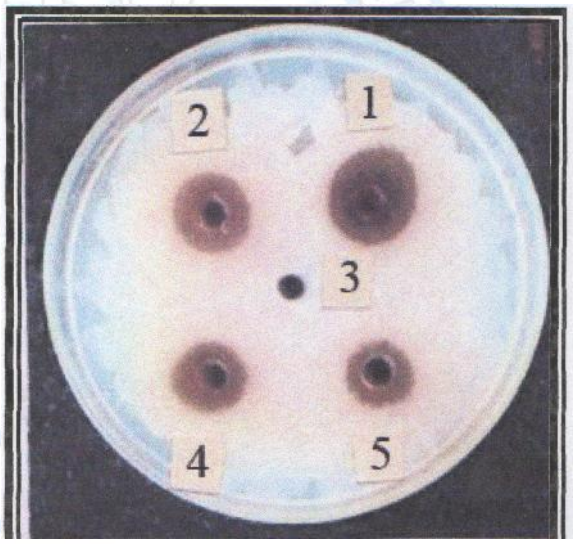

d

Figure-1-Effect of M.chamomilla flowers water extract on the growth of :

a-S.aureus,b-B.ceries ,c-E.coli ,d-Ps.aeruginosa.

1=40mg/ml,2=20mg/ml,3-control(P.B.S),4-=10mg/ml,5=5mg/ml

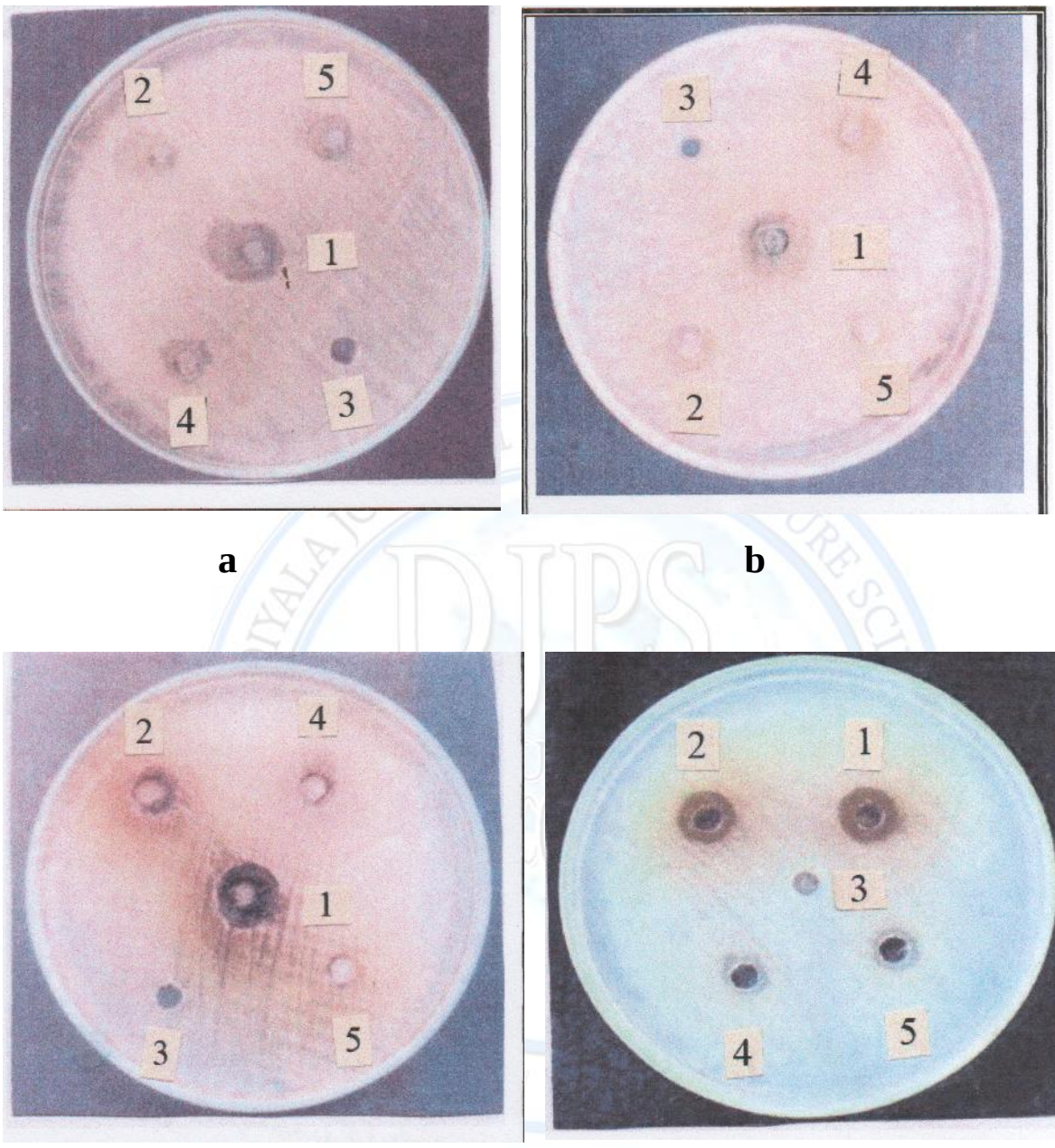

Figure-2-Effect of M.chamomilla flowers ethanolic extract on the growth of:

a-S.aureus,b-B.ceries,cE.coli,dPs.aeruginosa

1-=40mg/ml,2=20mg/ml,3=control(P.B.S),4=10mg/ml,5=5mg/ml

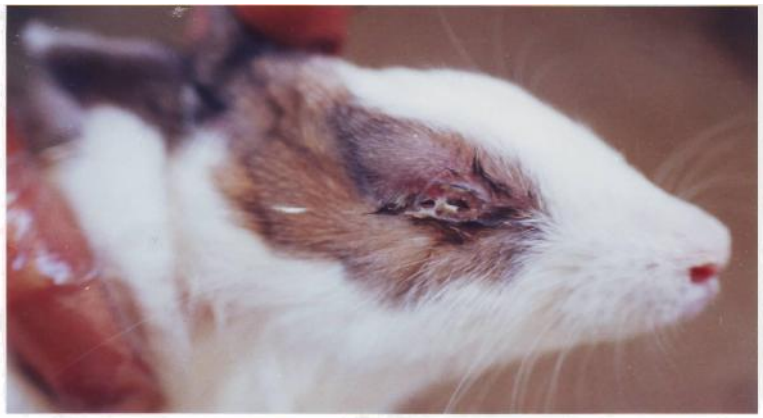

Figure-3-Rabbit eye injected with S.aureus and P.B.S.(as acontrol).

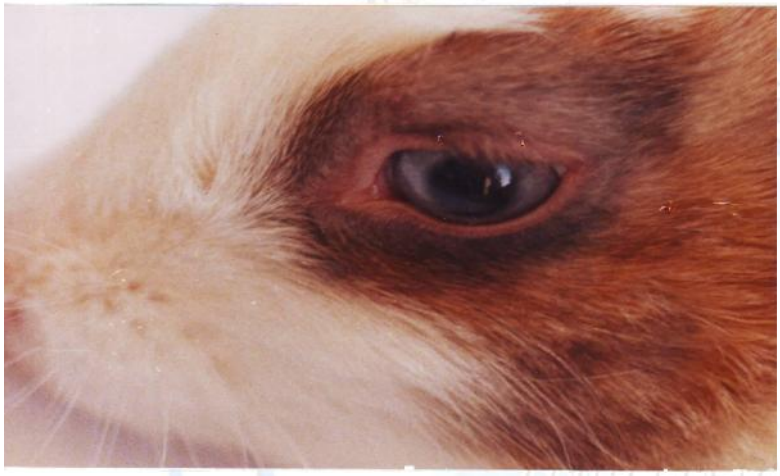

Figure-4-Rabbits eye after administration of ethanolic extract of chamomile at concentration 40 mg/ml.

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