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Antibacterial Activity of Water and Alcoholic Crude Extract of Flower *Achillea millefolium*

Rasha N. Hasson
Department of Biology
College of Education for Girls
Mosul University

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ABSTRACT

The aim of this study was to determine the antimicrobial effect of yarrow and it is flower extract on certain microorganisms. The ethanolic (alcoholic) extract of the flower and aqueous (water) extract was tested against: *Pseudomonasa aeruginosa, Salmonella enterica enterica (Typhimurium), Shigella flexneri, Micrococcus luteus, Staphylococcus aureus* and *Enterococcus faecalis.* Using the diffusion test, the alcoholic and aqueous flower extract at 200 mg /ml possessed abroad spectrum of antimicrobial activity against all tested microorganisms at different conc., alcoholic extract had the greatest inhibitory effect (diameter of inhibition zone) against *Pseudo. aeruginosa* 30 mm, *Staph. aureus* 24 mm and *Micro. lutues* 23 mm, While the aqueous extract slightly inhibited *Pseudomonasa aeruginosa* and *Micrococcus luteus* 12mm and 10 mm respectively compared with standard antibiotic Ciprofloxacin 26 mm, 22mm and 22mm but *Entero. faecalis*, *S. enterica enterica (Typhimurium)* and *Shig. flexneri* show high resistance for both extract 8 mm, 0 mm, 9 mm, 12 mm and 8 mm, 10 mm but sensitivity for Ciprofloxacin 23 mm, 30 mm and 31 mm. Minimum inhibitory concentration (MIC) were determined for alcoholic extract against sensitivite microorganisms: *Pseudo. aeruginosa* 50 mg/ml, *Staph. aureus* 100 mg/ ml and *Micro. luteus* 100mg/ ml. It could be concluded that the alcoholic extract of yarrow flower have considerable antimicrobial effect on some pathogenic which cause infection.

Keywords: *Achillea millefolium*, antimicrobial activity, ethanol extract, aqueous extract, yarrow, antibiotic.

الفعالية المضادة للجراثيم للمستخلص المائي والكحولي الخام لأزهار الاخيلية (ام الف ورقة)

الملخص

هدفت الدراسة إلى إظهار فعالية الاخيلية بوصفها مضاداً للجراثيم وتحديد تأثير مستخلصات الأزهار في تثبيط نمـو جـراثيم معينـة اذ أختبـر المـستخلص الكحـولي والمـائي لأزهـار النبـات المـضاد للجـراثيم: *Pseudomonasa aeruginosa*، *Salmonella enterica enterica(typhimurium)*، *Shigella flexneri*، *Micrococcus luteus*، *Staphylococcus aureus* و *Enterococcus faecalis* بطريقــة

الانتشاربالاقراص، أظهر المستخلص الكحولي والمائي بتركيز 200 ملغم/سم^3 طيفاً واسـعاً مـن الفعاليـة المضادة لنمو جميع الجراثيم المستخدمة في الاختبار وبشكل متفاوت فكان المستخلص الكحولي الأكثر تاثيرا اذ كان (قطر منطقة التثبيط) لكل من الجراثيم :*Pseudo. aeruginosa* 30ملم، *Staph. aureus* 24ملم و *Micro. luteus* 23ملم أما المستخلص المائي فقد أظهر تـأثيرا" أقـل و بـشكل ملحـوظ علـى الجـراثيم *Pseudomonasa aeruginosa* و*Micrococcus luteus* 12ملم و10ملم على التـوالي مقارنـة بالمـضاد الحيوي القياسي Ciprofloxacin: 26ملم، 22ملم و 22ملم ولكن الجراثيم *Entero. faecalis*، *S. enterica (Typhimurium)* و *Shig. flexneri* أظهرت مقاومة عالية لكل من المستخلصين اذ كانت اقطار التثبيط 8ملم و0ملم، 9ملم و12ملم و 8ملم و10ملم فيما أظهرت هذه الجراثيم حساسية عالية للمضاد الحيوي Ciprofloxacin وباقطارتثبيط بلغت 30ملم ، 30ملم و 31ملم لهذه الجراثيم على التوالي. تم تحديد التركيز المثبط الأدنى (MIC) للمستخلص الكحولي ضد الجراثيم *Pseudo. aeruginosa* وكان 50مليغرام / سـم^3، *Staph. aureus* 100مليغرام/ سم^3 و*Micro. luteus* 100 مليغرام / سم^3. يمكن الاستنتاج بان المستخلص الكحولي لازهارالاخيلية كان له تأثير فعال مضاد لنمو بعض الممرضات التي تسبب التهابات.

الكلمات الدالة: أخيلية أم ألف ورقة، الفعالية المضادة للجراثيم، مستخلص كحولي، مستخلص مائي، عشبة الجروح، مضاد حيوي.

INTRODUCTION

Plants, a source of medicinal compounds have continued to play a dominant role in maintenance of human health since ancient times. The World Health Organization estimates that plant extracts or their active constituents were used as folk medicine in traditional therapies of 80% of the world population (Anonynous, 1993). Over 50% of all modern clinical drugs are of natural origin (Baker *et al.*, 1995). Iraqi People have traditionally used a number of plants species for treatment of infections disease and various ailments (Najla and Awaz, 2009).

The treatment of infectious diseases with antimicrobial agents continues to present problems in modern day, medical studies showed a significant increase in the incidence of side effects and the resistance that pathogenic microorganisms build against several antibiotic (Kunin, 1993; Finch, 1998; Iwu, 1999; Nascimento, 2000). However attention has been paid to plant and biologically active compounds isolated from them used in herbal medicine such as alkaloids, flavonoids, isoflavonoids, tannins,glycosides and phenolic compounds (Evans *et al.*, 2002; Romero, 2005).

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm its activity (Nair *et al.*,2005). The effect of plant extracts on microorganisms have been studied by a number of researcher in different parts of the world (Mahansen, 1996 ; Kivcak *et al.*, 2002 : Uzan *et al.*, 2002 : Ates and Erdogrul , 2003

;Barbour *et al.*, 2004; Kirbag *et al.*, 2005 ; Sengul and Ogutcu, 2005; Dulger *et al*., 2005; Kumar *et al.*, 2006 ; Mathabe *et al.*, 2006).

Achillea Millefolium L. (Yarrow) is flowering plant in the family Achillea Asteraceae grows in Europe, North America and Asia. a number of species were used as garden omamentals. The aerial parts of yarrow have along history of traditional herb medicine in veterinary medicine (Ludwing, 1996; Hirti, 2000). Preparation in the form of decoctions or fresh juices have applied against anorexia, stomach, cramps, flatulence, gastritis, enteritis, internal and external bleeding (bloody cough, nosebleed, and menstrual bleeding, bloody urine), wounds, sores, skin rash as well as dog and snake bite sites. Its name is derived from Achilles (means a mythical character), who carried it with his army to treat the wounds of his fellow soldiers during Trojan war, its specific name means (a thousand leaves) and refers to its shape like bird feather. This medicinal utillsation is also reflected in some of its common name: wound wort A, nosebleed, blood wort, sanguinar, herb milltaris and knights milfoil (Chandler *et al.*, 1982).

The genus *Achillea* consists of 140 perennial herbs native to the northern hemispheres. Yarrow (fig. 1) aerial parts of the *Achillea* which has the widest range of herb applications in the world, it contains a volatile oil rich in sesquiterpene lactones and alkaloids (Muller *et al.*, 1994). *In vitro* studies yarrow flower extract showed its antimicrobial activity to be more effective against certain microorganisms than yarrow leaves and stems (Chandler *et al.*, 1982; Bisset, 1994; Pattnak, 1997; Nemeth and Bernath, 2008).

Fig.1: *Achillea Millefolium* (aerial parts)
(Chandler *et al*., 1982)

Many plants in Mosul city are familiar and widely used medicinal applications or have the potential to cure some diseases. Yet, these plant do not receive enough assessment therefore this study was conducted to determine the antibacterial activity of yarrow flower against some gram positive and gram negative bacteria.

MATERIAL AND METHOD

Plant: Dried yarrow flower were collected from Mosul herbal herbarium and identified according to the relevant literature (The complete medicinal herb) by herbalist in College of Agriculture, Mosul University (Penlope, 1999).

Test microorganisms: Six Bacteria: *Pseudo. aeruginosa, S. enterica enterica (Typhimurium)*(Tindall *et al.*, 2005; WHO, 2010), *Shig. flexneri, Micro. luteus, Staph. aureus* and *Entero. faecalis* provided by Mosul University College of Science, Department of Biology, Identification of bacterial species was confirmed using API *Staph*. and API Enterobacteracea (Collee *et al.,* 1996), The bacteria were isolated from clinical specimens.

Extract preparation: 10 gm of dried powder of yarrow flower was suspended in 150 ml of distilled water or absolute ethanol stirred on magnetic stirrer without heating until mixing well. then the mixture was left in refrigerator at $4C^{o}$ for 24hr after that both infusions were filtered through many layers of gauze and by using Whatman No.1 filter paper, after drying the two samples of dried extract weighted and stored at 4 C^{o} in dark bottles with aluminum foil to prevent negative effect of light (Al – Joboory and Al – Rawi,1994). Two sample stock solutions were prepared by suspending 1 gm of dried extract in 5 ml of distilled water or dimethyl sulfoxide for aqueous and alcoholic extract respectively 200 mg/ ml, then aqueous stock solution was sterilized by membrane filter o.22µ but alcoholic stock solution was sterilized by pasteurization at 62 C^{o} for 10 min. (Al-Neman ,1998). Then tested against all microorganisms, compared with standard antibiotic Ciprofloxacin 5µg/ml, the filter paper disks were impregnated into extract and antibiotic solution (Collee *et al.,* 1996).

Preparation of microbial cultures: The six bacteria were inoculated on nutrient broth incubated for 24hr., bacteria were inoculated in sterile Mueller Hinton agar (10^{8} CFU /ml). Discs were applied on the surface agar medium by pressing slightly Petri dishes and then incubated at $35C^{o}$ for 24hr. at the end of period, inhibition zones were measured in millimeters (Collins and Lyne, 1987).

Determination of MIC: the MIC for alcoholic extract showed highest antimicrobial effect, Dilution were prepared at concentration 150mg/ml, 100mg/ml, 50mg/ml and 25mg/ml for sensitive microorganisms (Atlas *et al*., 1995).

RESULTS AND DISCUSSION

In vitro antimicrobial activity of the aqueous and alcoholic extracts of yarrow flower 200 mg/ml was tested against three species of Gram –negative bacteria (*Pseudo. aeruginosa, S. enterica enterica (Typhimurium) and Shig. flexneri*) and three Gram – positive species (*Micro. luteus, Staph. aureus and Entero. faecalis*) compared with standard antibiotic ciprofloxacin 5µg/ml was shown in Table.1 and Fig.2.

Table 1: Antibacterial activity of yarrow flower extract.

Materials / **Microorganisms**	**Alcoholic (200 mg/mL)**	**Aqueous (200 mg/mL)**	**Ciprofloxacin (5 µ g /mL)**
	Diameter of zone of inhibition (mm)		
Salmonella enterica enterica (typhimurium)	9	12	30
Shigella flexneri	8	10	31
Pseudomonas aeruginosa	30	12	26
Staphylococcus aureus	24	-	22
Enterococcus faecalis	8	-	23
Micrococcus luteus	23	10	22

(-): No inhibition zone.

The alcoholic and aqueous flower extract of yarrow possessed a board spectrum of antimicrobial activity using the Kirby – Bauer disk diffusion test against all tested microorganisms at different raties, alcoholic extract had the greatest inhibitory effect (diameter of zone of inhibition) against *Pseudo. aeruginosa* 30 mm (1), *Staph. aureus* 24mm (2), and *Micro. lutues* 23 mm (3), while the aqueous extract slightly inhibited *Pseudomonasa aeruginosa* and *Micrococcus luteus* 12 mm and 10 mm respectively compared with standard antibiotic Ciprifloxcain 5 µg/ml: 26 mm, 22 mm and 22 mm but *Entero. faecalis*, *S. enterica enterica (Typhimurium)* and *Shig. flexneri* show high resistance for both alcoholic and aqueous extracts 8mm, 9mm, 8mm, 0mm , 12mm, 10mm compared to the sensitivity for ciprofloxacin : 23m, 30mm and 31mm, the results also showed that the most sensitive organism to both extracts was *Pseudo. aeruginosa* and the least sensitive organisme *Entero. faecalis*, then according to the results of dilution of alcoholic extract, the minimum inhibitory concentrations (MIC) sensitive microorganisms : *Pseudo. aeruginosa* (MIC=50 mg/ml), *Staph. aureus* (MIC=100 mg/ml) and *Micro. luteus* (MIC=100mg/ml).

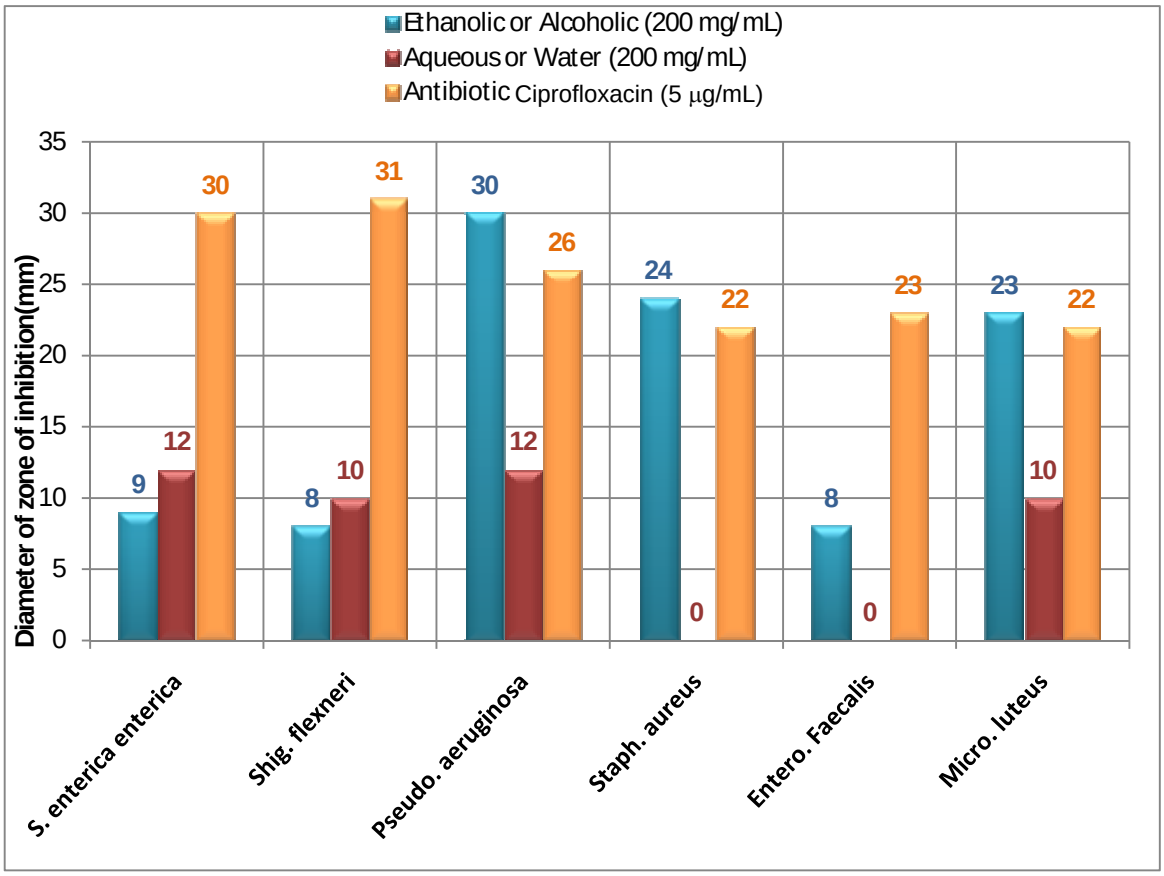
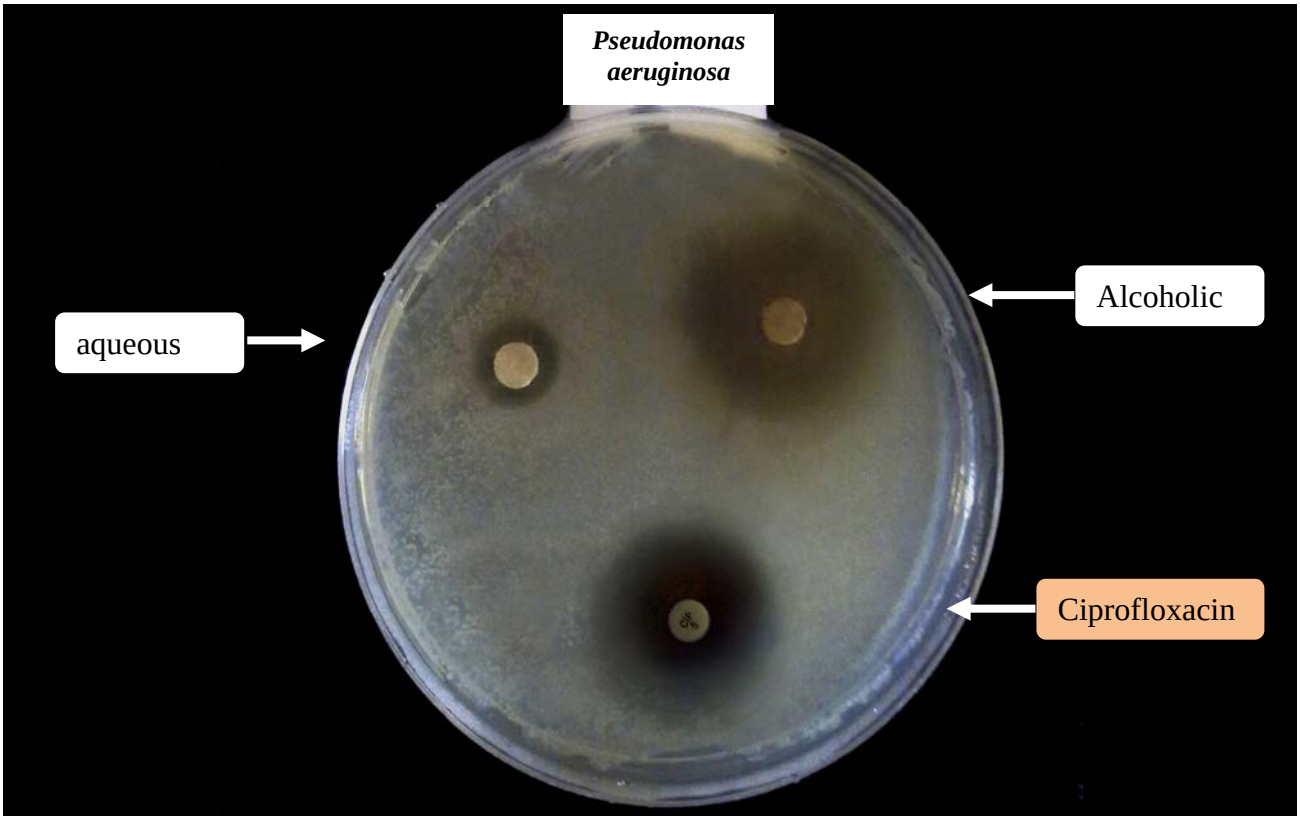
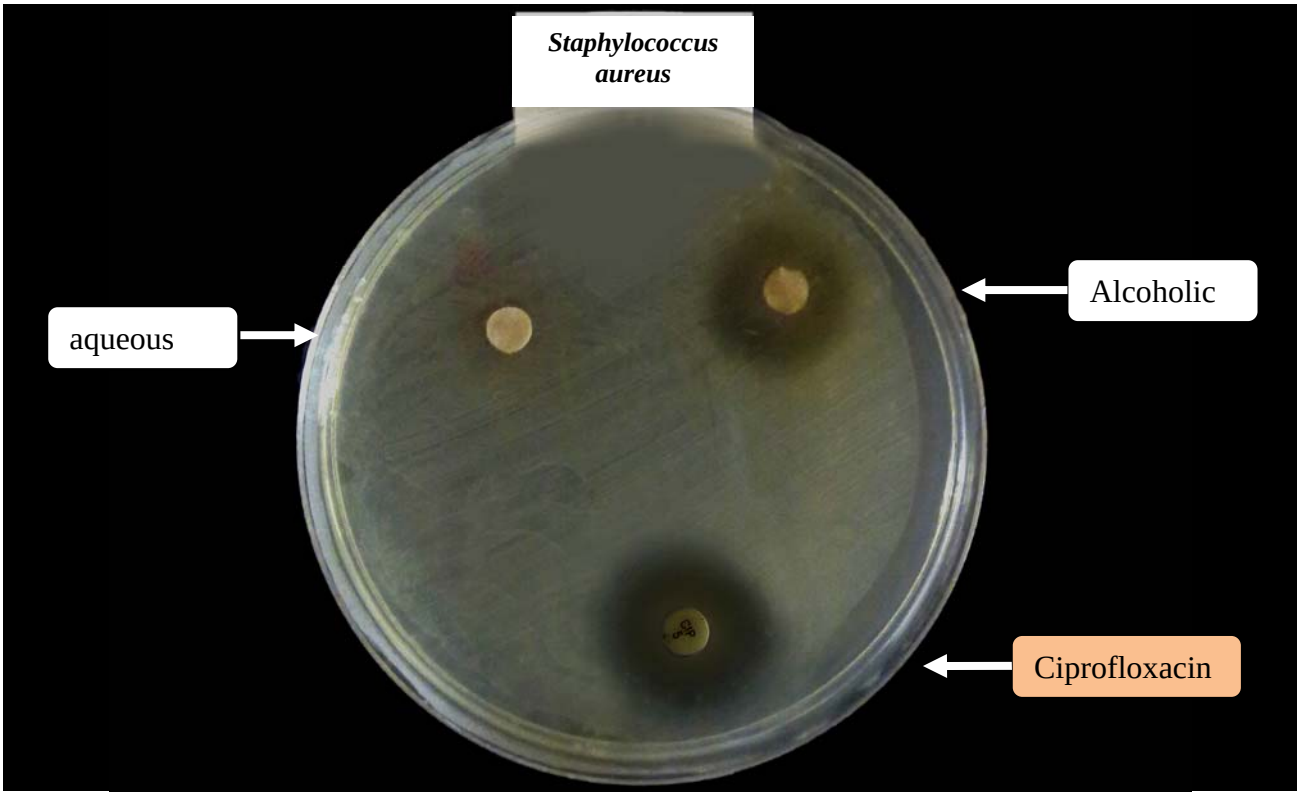


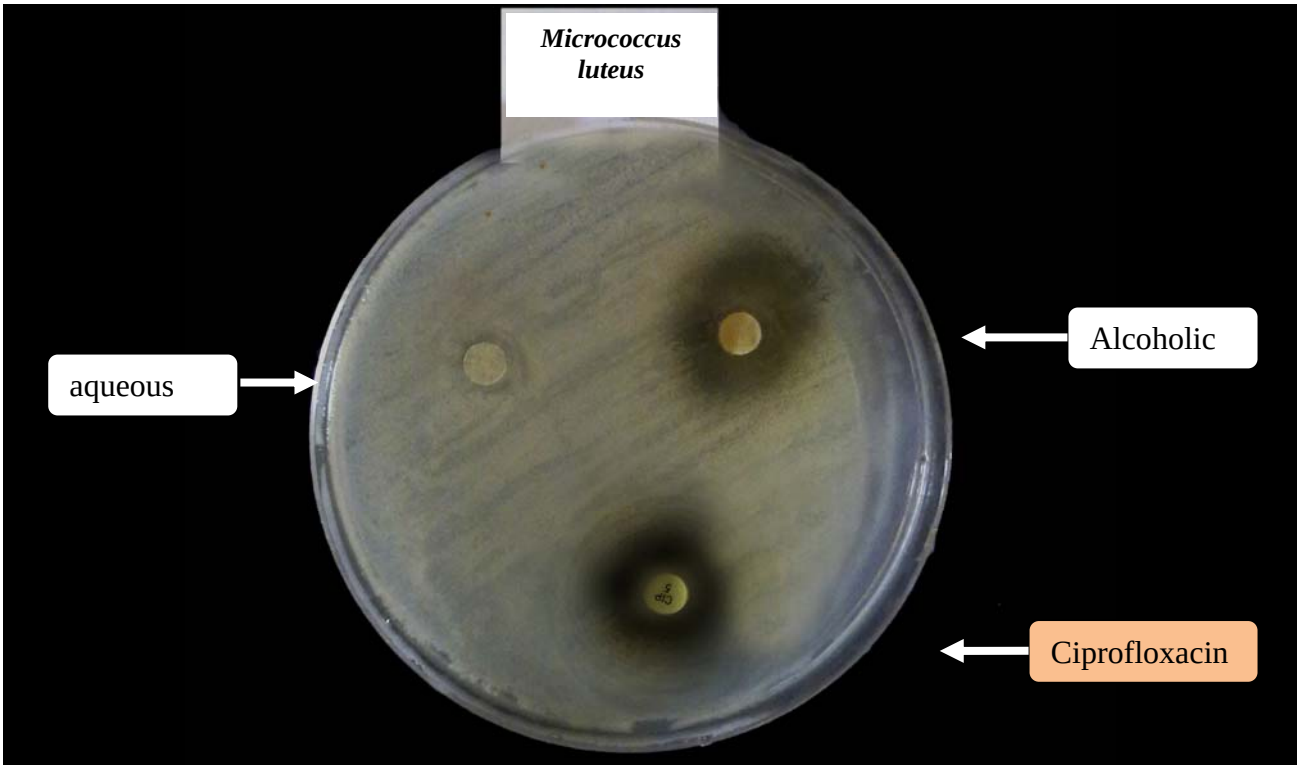
Fig. 2: Alcoholic and aqueous extract comparison with Ciprofloxacin.



Pic. 1 : *Pseudomonas aeruginosa*



Pic. 2 : *Staphylococcus aureus*



Pic. 3 : *Micrococcus luteus*

The result of this study agreed with recent investigation which have also pointed out to the notable effect of extracts of aerial parts of yarrow as it showed a board spectrum antimicrobial activities against *Staph. aureus*, *E. coli*, *K. pneumoniae*, *A. niger* and *C. albicans* (Candan *et al*., 2003 ; Skoeibusic *et al*., 2004; Radulovic, 2005).

It could be concluded that the alcoholic extract of yarrow flower have considerable antibacterial effect on some pathogenic microorganisms which cause infections. Therefore, befor application of these extracts as antimicrobial agents it must be evaluated *in vivo* and clinically.

CONCLUSION

On the light of these finding and other reports in this field, it could be concluded that:

Yarrow flower extract have potential antibacterial effect against certain micro-organisms which cause infections.

Possible benefits from other herbs with antibacterial activity. Further large – scale, well - designed clinical trails are required to prove more conclusive proof of their efficacy, the problem of microbial resistance is growing and the out look for use antimicrobial drugs in future is still uncertain, therefore, action must be taken to reduce this problem, control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs, either synthetic or natural. The aim is to offer appropriate and efficient antibacterial drugs to patient.

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