Study the inhibitory effects of aqueous and alcoholic extracts of Melia azedarach L. on some bacterial strain.

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Abstract

The current study aimed to preparation of an aqueous and alcoholic extracts of Melia azedarach L.leaves .The antimicrobial activity of these extracts was tested against four strains of bacterial isolates, two of them were Gram-positive, namely Staphyloccocus aureus NCTC6571 standard strain and Staphyloccocus aureus strain from some clinical patients. And other were Gram-negative, namely Escherichia coli ATCC25922 a standard strain and Pseuedomonas aeroginosa clinical strain isolated from some patients. Drug sensitivity test to clinical strains was also tested toward Psuedomonas aeroginosa , Staphyloccocus aureus) to Ciproflaoxacin, Cefoxitin , Strepomycin , Cefoxitin . The clinical bacterial strain Pseuedomonas aeroginosa showed resistance 100% to all antibiotics used in the experiment, while the Staphyloccocus aureus was sensitive to four antibiotics: Ampicillin, Ciproflaoxacin, Ampicillin, and Strepomycin.

The current study showed the superiority of the alcoholic extract in

inhibiting standard and clinical strains, including antibiotic resistance, while the aqueous extract did not show any

inhibitory activity against any of the clinical bacterial, and cytotoxicity was tested using human red blood cells(RBC) , where the results showed the absence of any Cellular toxicity for all concentrations used in the experiment, so the current study recommends the possibility of using the alcoholic extract of the rosary plant as drug alternatives after conducting cytotoxicity tests and drug dose (LD50) on the mentioned extract.

Key words : Melia azedarach L., aqueous extracts , alcoholic extracts, antimicrobial activity , bacterial strains , drug alternatives

Introduction

The use of medicinal plants for their antimicrobial action has been the subject of research by many workers and many works have been carried out in this field recently , to discover new antimicrobial drugs of

plant origin . It is recognized that in some developing countries , plants are the main medicinal source to treat infectious diseases(Sofowora, 1984 ; Oudhia,2001) .

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological test ,and a substatial number of new antibiotics introduced on the market are obtained from natural sources .(Mothana and Lindequist,2005). Melia azedarach L. (meliaceae) is one of the most useful traditional medicinal plant like Azadirachta indica is an evergreen tree (Schmuttere ,1995).and it introduced in south of Iraq Sibahbah and Zanzalakht and used some part as a cure for eye diseases and stomachache (Al-Rawi and Chakravart ,1964). and it was introduced to Egypt from Sudan around (Awad,1990) .

It is old tree in Egypt with reputed value for it is antifungal properties (Bina et al., 2004) , Neycee et al.,(2012)reported that M. azedarach aqueous leaf extract was a good inhibitor of Bipolaris micropus but with little or no effect on F. oxysporum .The antibacterial potential of M.asedarach L. was tested using crude leave , flower and fruit- seed extracts against pathogenic bacterial strains (Abdul Viqar et al.,2008).

The aim of this paper was study the physiochemical properties and the antimicrobial activity of crude extracts on the growth of some clinical bacteria, with determination of antibiotics susceptibility testing, The minimum inhibitory concentration (MIC).dosage of extracts against each bacterium was also evaluated and calculated.

Material and Methods

Preparation of extracts

1-Aqueous extracts

The air dried fine plant leaves (25g) were infused in (250 ml) distilled water until complete exhaustion. The extract was then filtered using Whatman No.1 filter paper and the filtrate was evaporated in vacuo and dried in room temperature Ahmad et al (1998).The final dried sample were stored in labeled sterile bottles and kept at -20 °C.

2- Ethanol extracts : The plant extract was prepared by following Ahmad et al (1998).procedure with some modifications .mature leaves M . azedarach measured amount of powdered material was soaked in alcohol (95% ethanol) The material was filtered with filter paper Whatman No.1 after one the filtrate was concentrated in a rotary evaporator. The remaining alcohol was evaporated in an incubator at 60 ˚C till maximum alcohol was evaporated .

3-Phytochchemical Analysis

The estimation of various phytoconstituents i.e. Alkaloids , Saponine , Tannins , Steroids , Flavonoids and Glycosides by using several tests such as :

a- Alkaloid test, Dragendroff , Mayer .

b- Tannins test , the dark green color indicates the presence of tannins.

c- Flavonoids test : use alcoholic KOH (5N)

d- Saponins test : use mercuric chloride (5%)

e- Glycosides test: use Bendicts test.

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Microorganisms

The pathogenic strains of, Pseudomonas aeroginosa* , Staphylococcus aureus* for antibacterial test were used . These strains were obtained from some patients .

Antimicrobial Sensitivity Test

The activity of plant material was tested by the well diffution method (NCCLS, 1997) .

Determination of minmum inhibitory concentration (MIC)

The MIC was evaluated on plant extracts that showed antimicrobial activity . MIC values were studied for microorganisms which were determined by micro dilution broth methods (Bassole et al., 2003).

compound Test	Formation Orange precipitate	Formation light brown precipitate	Formation white precipitate

Table (2) the qualitative chemical tests for the isolated compound of Melia azedarach L.

No	Photochemical	Result
1	Alkaloid	+
2	Crude glycosides	+
3	Flavonoids	+
4	Tannic acid	+
5	Saponins	+

Table (1) the qualitative chemical analysis for the isolated compound of Melia azedarach L.

No	Antibiotics	Interpretation	
		Psuedomonas aeroginosa*	Staphylococcus aureus*
1	Ampicillin	R	S
2	Ciproflox acin	R	S
3	Cefoxitin	R	R
4	Streptomy cin	S	S
5	Cefazolin	R	S

Reagent	Dragendroff	Wagner	Mayer
Alkaloid	+	+	+

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Psuedomonas aeroginosa* and **Staphylococcus aureus***

*Resistant (R) Sensitive(S)
* clinical bacterial isolates

Table (4) the antibacterial activity of the crude extracts of Melia azedarach L. and the minimum inhibitory concentration (MIC).

Bacteria strains	**Inhibition zone (mm)****					
	Aqueous extracts			Ethanol extracts		
	ml/mg			**ml /mg**		
	125	250	500	125	250	500
Staphylococcus aureus NCTC 6571	6	6	6	6	10	26
Escherichia coli ATCC 25922	6	6	6	6	6	15
Staphylococcus aureus *	6	6	6	6	10	15
Psuedomonas aeroginosa*	6	6	6	6	10	10

**Mean of three value each number

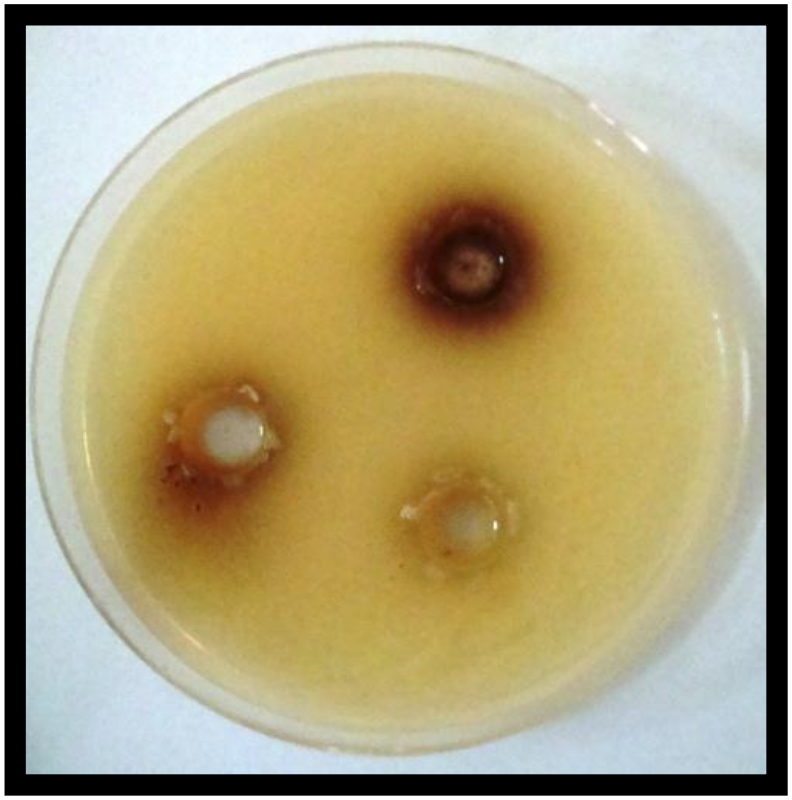
Figure(1) antibacterial activity of ethanol extract in strain

Figure(2) antibacterial activity of ethanol extract in 250, 125 , 62.5 µg/ ml against E.coli strain

Figure(3) antibacterial activity of ethanol in 250, 125 , 62.5 µg/ ml against **Staphylococcus aureus** strain

Results and Discussion

The medicinal value of plants are very important and widely available resource



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for primary healthcare and complementary healthcare systems(Pankaj et al., 2008). The well diffution method was used to determine the antibacterial agar effect of the aqueous and ethanolic extract of Melia azedarach L. The ethanolic extracts were found to be more effective than aqueous extracts Table (1).

The antibacterial activity of crude extracts determined by using Agar Diffusing Method (Anonymous,1996).The results , in table [5], showed that the crude ethanolic extracts of Melia azedarach has good antibacterial activity against gram positive and gram negative bacteria , including Staphylococcus aureus NCTC 6571 and Escherichia coli ATCC25922 and Staphylococcus aureus* and Psuedomonas aeroginosa* as a clinical bacterial isolates showed relatively in its effectiveness by resistant for antibiotic drug, Cefazolin , Cefoxitin Ciprofloxacin ,Streptomycin that was multi drug resistant (MDR) Staphylococcus aureus resistance to methicillin and other similar antibiotics is due to the altered structure of penicillin binding proteins. This mutation is caused by resistance genes that are carried in the staphylococcal cassette chromosome (SCC) mec, a mobile genetic element. The cassette encodes for an insertion sequence element, recombinases and regulatory genes. Five versions of SCCmec have been identified, each of which confers resistance to slightly different set of agents.

The results of the effectiveness of plants , this my due to the combined action of numerous of chemical compounds such as alkaloids and glycosides phenols ,flavonoids ,tannins, coumarins these result inhibited the growth of E.coli and Staph.aureus . The results were also supported by Hymete et al ., (2005) thy reported that flavonoids compounds with other active compound also supported by Kher et al., (1984) they reported that 10% chloroform extracts imported inhibitory effects against Staphylococcus aureus and Escherichia coli .

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وذلك في تثبيط العزلات القياسية والمرضية بما فيها المقاومة للمضادات الحيوية في حين لم يظهر المستخلص المائي اي فعالية تثبيطية تجاه اي من العزلات الجرثومية السريرية ،كما جرى اختبار السمية الخلوية باستخدام كريات الدم الحمراء للإنسان حيث اظهرت النتائج عدم وجود اي سمية خلوية لجميع التراكيز المستخدمة في التجربة ، لذا توصي الدراسة الحالية في امكانية استخدام المستخلص الكحولي لنبات السبحبح كبدائل دوائية بعد اجراء اختبارات السمية الخلوية والجرعة الدوائية (LD50) على المستخلص المذكور.

الكلمات المفتاحية : نبات السبحبح Melia azedarach L. ، مستخلص مائي ، مستخلص كحولي ، فعالية ضد ميكروبية ، عزلات جرثومية ، بدائل دوائية .

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دراسة الفعالية الضد مايكروبية للمستخلص المائي والكحولي لنبات السبحبح تجاه بعض العزلات الجرثومية .

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المستخلص

تضمنت الدراسة الحالية تحضير مستخلص مائي و مستخلص كحولي لاوراق نبات السبحبح Melia azedarach L ، اذ اختبرت الفعالية الضد ميكروبية لهذه المستخلصات تجاه اربعة انواع من العزلات الجرثومية توزعت اثنتان منها موجبة لصبغة كرام وهما Staphyloccocus aureus NCTC6571 عزلة قياسية و Staphyloccocus aureus عزلة سريرية عزلت من بعض المرضى ، واثنتان من العزلات كانت سالبة لصبغة كرام وهما Escherichia coli ATCC25922 عزلة قياسية و Psuedomonas aeroginosa عزلة سريرية عزلت من بعض المرضى ، كما جرى اختبار الحساسية الدوائية تجاه العزلات المرضية السريرية (وPsuedomonas aeroginosa ، Staphyloccocus aureus) تجاه كل من المضادات الحيوية Ampicillin, Ciproflaoxacin,Cefoxitin,Strepomycin,Cef oxitin وقد اظهرت العزلة الجرثومية السريرية Psuedomonas aeroginosa مقاومة بنسبة 100% لجميع المضادات المستخدمة في التجربة ، في حين كانت العزلة المرضية السريرية Staphyloccocus aureus حساسة تجاه اربعة من المضادات الحيوية Ampicillin, Ciproflaoxacin,Ampicillin,Strepomycin ، اظهرت الدراسة الحالية تفوق المستخلص الكحولي

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