Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(4), 1001-1006 | ISSN:2251-6727

*Azadirachta indic*ia leaves extracts as antimicrobial effects against some different species of bacteria isolated from a burn patient's bodies.

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(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS

Azadirachta indica .Neem extracts .Staphylococcus aureus. Pseudomonas argenosa . resistant .*Staphylococcus aureus. Saccharomyces Boulardii.*

ABSTRACT

This study was conducted to determine the bacterial genera isolated from patients lying in the burn rooms at Ramadi Teaching Hospital, Burn Center. The isolated bacterial genera were several bacterial genera, from which the most resistant to antibiotics were selected, including the genera *Enterococcus faecalis, Pseudomomnas stutzeri, Staphylococcus epidermis, Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa*. *Staphylococcus epidermidis Staphylococcus hemolytica* The aqueous, annual and acetone extracts of the neem plant prepared at specific dilutions were used in the study. The highest effect was of the aqueous extract. The rate of inhibition against the bacterial genus Serratia marcescens was 0.9 cm for the concentration of 100 g/L of dry neem leaves, while the results showed the effect *of Saccromyces. bulardii* when incubated at 37 degrees Celsius at 72 hours had the highest effect on the growth of the pathogenic species under study, if the highest effect reached 1.3 cm against *Staphyloccus epidermidis*, while the lowest rate of infection was due to the resistance mechanisms possessed by the yeast in addition to the various substances contained in the neem plant.

Introduction

All deaths following thermal injuries are related to infection. Burn wounds are highly susceptible to infection and this is a major problem in the management of burn victims. Infected burn victims tend to stay longer in the hospital and have a higher mortality rate due to sepsis. The pathogenesis of colonization, infection and invasion of the burn wounds is related to the disruption of the normal skin barrie [1].

Antibiotics provide the basis for the fungal and bacterial infections therapy. The indiscriminate use of antibiotics in human and veterinary healthcare has led to the emergence of multi-drug resistant (MDR) strains of different groups of microorganisms. MDR strains have made antibiotics ineffective for the treatment of infectious diseases caused by such bacteria. Scientists are looking for alternative antibiotics from medicinal plants [2]. The screening of plants for medical purposes is an effort to find new, safer, and possibly more effective alternatives to synthetic drugs. Green medicines are safe and dependable in contrast with expensive synthetic drugs that have undesirable side effects and beneficial effects [3, 4]. Plants have been in use in traditional medicine since long time but are still understudied. *Azadirachta indica* (*A. indica)* is a native tree of India. It is a tropical evergreen plant with a wide adaptability. *Neem* tree is widely grown and cultivated throughout the country especially in semi-arid conditions. *Neems* have many medicinal properties, including antibacterial and antifungal. Probiotics have demonstrated an ability to prevent and treat some infections [5, 6] . Saccharomyces Boulardii non-pathogenic yeast used as a preventive and therapeutic agent for the treatment of a variety of diseases. Additional controlled studies are needed to clearly define the safety and efficacy of probiotics. *Saccharomyces boulardii* was discovered by Henri Boulard in 1920 in IndoChina*[7]*. It is a yeast isolated from the skin of Lychees grown in Indochina. The name used most commonly is probiotic. It's defined as a live microorganism or microbial mixture administered to improve the host animal's microbial balance. Saccharomyces boulardii (Sb) is a non-pathogenic yeast used for many years as a probiotic agent to prevent or treat a variety of human gastrointestinal disorders. Probiotic is derived from Greek and means for life. Lactobacillus species and Bifidobacterium species are the most common probiotics [8].

Materials and Methods

Azadirachta indica (Neem) leaves extracts have antimicrobial properties against selected pathogens. This was a laboratory In vitro based study conducted to evaluate

Journal of Chemical Health Risks

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JCHR (2023) 13(4), 1001-1006 | ISSN:2251-6727

the antimicrobial effects of *Azadiracachta indica (* leaves extracts on selected pathogens in the lab.

Preparation of extracts

Then the solutions were filtered and the aqueous solution was heated until it boiled for 30 minutes, while the alcoholic and acetone solutions were left in the incubator for 24 hours. Water at a temperature of 40 degrees Celsius for 24 hours, after which samples were collected and used in the study Then the solutions were filtered and the aqueous solution was heated until it boiled for 30 minutes, while the alcoholic and acetone solutions were left in the incubator for 24 hours. Water extract at a temperature of 40 degrees Celsius for 24 hours, after which samples were collected and used in the study Different dilutions of 100%, 75%, 50%, and 25% were prepared by adding distilled water to the solutions.

Microbial strains

Antimicrobial activities of extracts of A. indica leaves were evaluated against clinical isolate of include S. aureus, E. coli, P. aeroginosa, and. All pathogens were obtained from ramadi hospital teachening with ramadi burn center [9].

Preparation of culture media (according to manufacturer instruction)

Mueller Hinton agar (MHA) media was prepared by suspending 38 g in 1000 ml of distilled water. Media was sterilized by autoclaving at 121℃ for 15 min and poured into sterile Petri plates at around 50ˊ C up to a uniform thickness of approximately 4 mm [10]

Preparation of different concentrations of the extracts.

The solvent of 10% DMSO was used to prepare stock solutions of methanol and acetone leaves extract with concentration of 80g/1000 ml. and then concentrations of 75%, 50%, and 25% of each extract were also prepared by the same solvent [11].

Antimicrobial assay

Antimicrobial assays were performed by agar well diffusion method. Plates were swabbed with cotton wool impregnated with the bacterial suspension containing 106 CFU/ML and allowed to dry. Five wells were bored on the surface of the agar media on each plate. The wells were filled with 100 µl solution of the extract at 100%, 75%, 50%, and 25%. The last well was filled with DMSO as negative control of each extract. A disk of appropriate antibiotics were used as positive control [12].

Second metabolism from Saccharomyces *bulardii*

second metabolism from *Saccharomyces bulardi* from preparation of sabroid broth by 20mg 1000ml and addition of 10000 coloni from yeast and incubate for 24 h, 48 h, 72 h and 96h and screened the effect on different species of burn bacteria [13].

Data analysis

Table 1. result of culture

Type of bacteria	Frequency	Percent
11-Enterococcus faecalis	**2**	**4%**
2- *Pseudomomnas stutzeri*	**7**	**14%**
6- *Staphylococcus epidermis*	**7**	**14%**
7- *Escherichia coli*	**10**	**20%**
9 *Serratia marcescens*	**10**	**20%**
Pseudomonas aeruginosa	**5**	**10%**
5-*staphyloccus epidermidis*	**5**	**10%**
10- *Staphylococcus hemolytica*	**4**	**8%**
Total	**50**	**100**

Table 2. The result of commercial antimicrobial disc measured by cm:

Antimicrobial discs used:	***Enterococcus faecalis***	***Pseudomomnas stutzeri***	***Staphylococcus epidermis***	***Escherichia coli***	***Serratia marcescens***	***Pseudomonas aeruginosa***	***staphyloccus***	***Staphylococcus hemolytica***

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JCHR (2023) 13(4), 1001-1006 | ISSN:2251-6727

							epidermidis	
imipenem **(ipm)**	R	3	1	2.7	2.5	2.5	3.4	2.5
Amoxicillin + clavulanic acid (amc)	R	1.5	1.5	1.5	R	1.4	1.3	1
Bacitracin (B)	R	R	R	1.2	R	R	1	R
AMIKACINE (AK)	R	R	2.1	1.8	1.8	2.6	2.5	0.5
Ceftriaxone (CRO)	R	1.4	1.5	1.7	2.5	2	2	1.5
Cefixime (CTX)	R	R	R	R	1	1.7	2	1
Cefalexin (CN)	R	R	2.4	2	2	2.5	3	2
Tobramycin (TOP)	1.8	R	1	2	1.7	2.7	3.4	2
Ciprofloxacin (CIP)	1.8	1.4	2.5	2.5	3	3.4	3.5	2.7
Meropenem (MEM)	1.8	1.6	1	3	3	3	2	2.6

Table 3. Effect of different concentrations of *Neem* acetone extract and methanol extract on the selected bacteria by measuring zone of inhibition in cm

Neem ethanol extract with Concentration in %	***Enterococcus faecalis***	***Pseudomomnas stutzeri***	***Staphylococcus epidermis***	***Escherichia coli***	***Serratia marcescens***	***Pseudomonas aeruginosa***	***staphylococcus epidermidis***	***Staphylococcus hemolytica***
100%	0.8	0.8	0.94	0.4	0.34	0.88	0.2	0.3
75%	0.6	0	0	0	0	0	0	0.4
50%	0	0	0	0	0	0	0	0
25%	0	0	0	0	0	0	0	0
Neem acetone extract with Concentration in %								
100%	0.8	0.8	0.6	0.8	0.8	0.8	0.9	0.9
75%	1.1	0	0	0.8	0	1.1	0	0
50%	0.6	0	0	0	0	0	0	0

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JCHR (2023) 13(4), 1001-1006 | ISSN:2251-6727

Aqueous extract of neem leaves in %	***Enterococcus faecalis***	***Pseudomomnas stutzeri***	***Staphylococcus epidermis***	***Escherichia coli***	***Serratia marcescens***	***Pseudomonas aeruginosa***	***staphyloccus epidermidis***	***Staphylococcus hemolytica***
100%	1.1	1.1	1	1.2	0.9	1.2	1	1.1
75%	0.9	0.8	0.9	0.8	0.8	0.8	0.9	0.8
50%	0.7	0.7	0.6	0.5	0.6	0.5	0.5	0.7
25%	0	0	0	0	0	0	0	0

Table 4. The result of second metabolism *Saccromyces bulardii with different time incubation*

Second metabolism *saccromyces bulardii*	***Enterococcus faecalis***	***Pseudomomnas stutzeri***	***Staphylococcus epidermis***	***Escherichia coli***	***Serratia marcescens***	***Pseudomonas aeruginosa***	***staphyloccus epidermidis***	***Staphylococcus hemolytica***
24 h	0.3	0.0	0.4	0.5	0.4	0.4	0.5	0.0
48 h	0.8	0.7	0.7	0.6	0.6	1.2	0.9	1.0
72h	1.2	1.2	1.1	1.1	0.9	1.2	1.3	1.1
96 h	1.2	1.4	1.2	1.1	1.2	1.2	1.3	1.2
120 h	1.3	1.4	1.2	1..2	1.3	1.1	1.5	0.5

Discussion

WHO reports that the world is coming into a post antibiotic era and most of current antibiotics will become inefficient. Antimicrobial compounds extracted from plants have great therapeutic potentials against microbes. They can help in aliment without unpleasant side effects. Research is under way to identify the effective and safe alternatives to current antibiotics from plant.(2)

The current study aimed to isolate and identify the bacteria associated with burn wounds and investigate the antimicrobial susceptibility pattern against a group of most commonly prescribed antibiotics. In total, 50 burn wound swabs were collected from burn patients admitted to the burn unit of AL-Ramadi Teaching Hospital in Ramadi City, Iraq. The swabs had been cultured on different media; the colonies were diagnosed based on the phenotypic and culture characteristics. The bacteria were identified through cultural characters and Gram staining diagnosed by VITEK® 2 Compact Automated Systems. In total, there were nine distinct bacterial isolations, of which, *Enterococcus faecalis*[4%]

Pseudomomnas stutzeri[14%]

Staphylococcus epidermis[14%]

Escherichia coli[20%]

Serratia marcescens[20%]

Pseudomonas aeruginosa was the most common pathogen [10%]

staphyloccus epidermidis[10%]

- *Staphylococcus hemolytica*[8%]

Enterococcus faecalis showed high resistance to most antibacterial. These results are consistent with what (Christopher.eat2014)

Christopher and his group found resistance to Enterococcus faecalis bacteria. There are several reasons for the resistance of this genus to many antibiotics, including Genomic exchange, Glycopeptide resistance, Regulation by host factors and Daptomycin resistance.

*Pseudomomomnas stutzer*i showed additional resistance to antibiotics. The study was also identical to what [14] and his group found that some bacteria are resistant to many antibiotics, including anti-bacteryacine with amkacine this is due to the fact that clinically important pathogens often contain mobile genetic elements. For secondary metabolites, bacteria are synthesized on the surface of bacterial ribosomes that function primarily in the cell envelope or inside the cell [15].

From Table 3, the results were similar to results of [16] in his study, he indicated the effect of aqueous and alcoholic neem leaf extracts. The antibacterial and antifungal activities of Neem extracts against food borne pathogenic bacteria and mycotoxigenic fungi were reported by Disc Diffusion Method [17]. The successful leaf extracts showed different abilities to inhibit the growth of the pathogenic bacterial and fungal strains. The ethyl extract inhibited bacteria with an inhibition zone of 12.16 mm against, ,muti

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JCHR (2023) 13(4), 1001-1006 | ISSN:2251-6727

species of bacteria The aqueous and ethanolic extracts inhibited and. *Azadirachta indica (Neem)* plant contains many antioxidant compounds that had an effective role in inhibiting bacterial species. The neem plant contains the compounds cinnamic acid ferulic acidnaringenin, taxifolin, kaempferol, and vanillin. , which could explain the involvement of Neem leaf extracts in many biological activities [18]. The cell reinforcing effect has been attributed to medicinal herbs which he referred to the researcher in his previously mentioned *studycereus, St. sciuri, staff. aureus, E. coli, and P. aeruginosa.* The aqueous and ethanolic extracts inhibited *P.s stutzeri* and S. enterica [19].

the age of the plant and the aqueous and ethanolic extracts isolated from it. The dilutions reduced the results of the effect on the bacterial species under study. The active compounds lost their effect at the mentioned dilutions and the results.

The study showed the effect of the acetone extract on the growth of the bacterial species under study, and it is consistent with [20] what and his group found, if he found the fungicidal and bactericidal in his study.

Properties of extracts from *Azadirachta indica (Neem* leaves either in vitro or in vivo trials to the presence of several antimicrobial active ingredients in leaves of neem tree such as desactylimbin,quercetin and sitosterol. Whereas other researchers explained this activity by the presence of active ingredients like triterpenes or the limonoids such as meliantriol, azadirachtin, desactylimpin, quercetin, sitosterol, nimbin, nimbinin, Nimbidin, nimbosterol and margisine and/or to different bitter substances such as alkaloids, phenols, resins, glycocides, terpenes and gums Lyer and Williamson attributed Antifungal properties of neem extracts to the inhibition in protease activity of dermatophytes induced by the neem organic extract [21].

The study showed that the effect of S. boulardii It was incubated for 72 hours at a temperature of 37°C against. The bacterial species against which the effect was examined, and this result was completely consistent with what was found by [22], which stated that the yeast mentioned above affects pathogenic bacteria through appeared to act by two main mechanisms: (i) production of factors that neutralized bacterial toxins and (ii) Modulation of the host cell signaling pathway in pro-inflammatory response during bacterial infection .from Table 4 shows the results of the effect against the bacterial species used in the study. The effect was close, which gives a clear idea that the mechanism of action was similar against all bacterial species [23].

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