



## ANTIBACTERIAL ACTIVITY OF *RICINUS COMMUNIS*: IN VITRO STUDY

\*<sup>1</sup>Hayder.M.K.Al-kuraishy; \*\*<sup>2</sup>Salah A.Al-weendy; \*\*\*\*<sup>3</sup>Ali K.Al-buhadilly, \*\*\*\*<sup>4</sup>Iman N.A.Al-bajajy, \*<sup>5</sup>Ali-I.Al-gareeb , \*\*\*<sup>6</sup>Ammar A. Ashoor Al-hafied

\*Department of of Pharmacology and medicine, College of Medicine, University of Al-Mustansiriya . Baghdad-Iraq

\*\*Department of microbiology, College of sciences. University of Baghdad. Baghdad-Iraq.

\*\*\*College of Medicine, University of Al-Mustansiriya. Baghdad-Iraq

\*\*\*\* Department of Pharmacology, College of Medicine,, University of Al-Mustansiriya. Baghdad-Iraq

<sup>1</sup> P.O. Box 14132, Baghdad, Iraq, Email:Hayder\_M36@Yahoo.com

<sup>2</sup> Salahw2008@yahoo.com, <sup>3</sup> P.O. Box 14132, Baghdad, Iraq

<sup>4</sup> P.O. Box 14132, Baghdad, Iraq, <sup>5</sup> P.O. Box 14132, Baghdad, Iraq

<sup>6</sup> P.O. Box 14132, Baghdad, Iraq

### Abstract

*Ricinus communis* herb produces significant antimicrobial activity particularly against Gram negative bacteria, in comparison with standard antibiotics. Four bacterial genera were selected two Gram negative (*Escherichia coli*, and *Pseudomonas aeruginosa*); and two Gram positive (*Staphylococcus aureus*, *Enterococcus fecalis*). These bacterial isolates were incubated and subsequently adding *Ricinus communis* extracts which were prepared as alcoholic and aqueous solutions .The MIC (Minimal Inhibitory Concentration)was determined for ten isolates of each bacteria . Results showed that the MIC of aqueous extracts ranged between 8-32mg/ml for all selected bacteria while the MIC of the alcoholic extract ranged between 8-16mg/ml. Moreover; the lowest MIC of alcoholic extract was 8mg/ml while the lowest MIC for aqueous extract was 16mg/ml. In conclusion, the alcoholic and aqueous extract generates specific MIC, but the alcoholic extracts produce more particular effects by lower MIC (8 mg/ml). Thus; a topical application of these extracts are useful as alternative antimicrobial remedy regarding the sensitive bacteria.

**Keywords:** antibacterial activity; ricinnis communis

### التأثير البكتيري المضاد للخروج:دراسة مختبرية

\*حيدر القرشي، \*صالح الدين الوندي، \*ايمان ناجي الباجي، \*علي البهادلي، \*علي الغريب، \*\*\*عماروهم

عاشور

\* قسم الطب الباطني والادويه السريري ، كلية الطب، الجامعة المستنصرية. بغداد-العراق

\*\* قسم علوم الحياة، كلية العلوم، جامعة بغداد. بغداد-العراق

\*\*\* كلية الطب، الجامعة المستنصرية. بغداد-العراق

\*\*\*\* قسم الادويه السريري، كليه الطب، الجامعه المستنصريه. بغداد-العراق

### الخلاصة

نبات الخروع له تأثير فعال ضد البكتيريا وخصوصا ضد البكتيريا السالبة لصبغة كرام عند مقارنته مع المضادات الحيوية القياسية. لهذا هدفت الدراسة الحالية لمعرفة التأثير البكتيري المضاد وتحديد التركيز المثبط الأدنى للمستخلص المائي والكحولي لبذور هذا النبات. لقد تم اختيار أربعة اجناس من البكتيريا فقد اختيرت اثنان موجبة (المكورات الذهبية والنتيروكوكس) واثنان سالبة (اشريجياكولاي والسيدومونس اريجنوسا) لصبغة كرام وبعدها تم حضنها في الوسط الملائم لكل بكتيريا وتم تحضير المحلول المائي والكحولي لبذور هذا النبات لمعرفة التركيز المثبط الأدنى. ولقد أظهرت النتائج بان التركيز المثبط الأدنى للمحلول المائي يتراوح بين ٨-٣٢ ملغ/ملييلتر لكل البكتيريا المختارة بينما التركيز المثبط الأدنى للمحلول الكحولي كان يتراوح بين ٨-١٦ ملغ/ملييلتر. في هذا البحث والدراسة المختبرية تبين بأن المحلول الكحولي والمائي اظهروا تأثيرات مختلفة وان المحلول الكحولي لهذا النبات له تأثير افضل على البكتيريا من المحلول المائي. لهذا فأن التأثير الموضعي لهذه المستخلصات تكون ذو فائدة كعلاج بديل اخذين بنظر الاعتبار البكتيريا الحساسة لهذه المستخلصات.

### Introduction

Many infectious diseases are known to be treated by herbal remedies all over the narration of mankind; the plants have long been used as remedies for treatment of many infections [1]. Also, plants still continues to be the major and exclusive sources of drugs for preponderance of the humanity population [2]. In recent years a number of studies dealing with the antimicrobial activity of plant extracts together with some plant constituents of potential significant medicinal activity have been reported [3]. A vast majority of population particularly those living in villages depend largely on herbal medicine, so scientific data on a good number of medicinal plant investigated has been well documented however, only very few drugs of plant origin could reach clinical uses and the national for mulary could not adopt even a dozen of plant medicines [4]. Therefore; a special efforts are needed for development of herbal drugs that have therapeutics utility. There are numerous herbal agents having different antibacterial, antifungal, anti-inflammatory and antineoplastics effects [5]. The most popular herbal in USA in 1996 were (in descending rank order) echinacea, garlic, ginseng, goldenseal, Mahuang, Psyllium [6]. These alternative medicinal substance are distinguished from similar botanical substances used in traditional medicine like Morphine and atropine [7].

The *Ricinus communis* is a plant species of the Euphorbiaceae also called castor oil plant [8]. Castor seeds have been found in Egyptian tombs dating back to 4000 BC and were used for lighting, body ointment and improving hair growth and texture [9]. The name *Ricinus* is a latin word for tick; the seed is so named because it has markings and a bump at the end which resemble certain tick, but the name castor oil

came from its use as a replacement for castoreum, a perfume base made from dried perineal gland of beaver [10]. The *R. communis* extract used since 2000 BC in India as local medicine for cathartic effects also for dermal fungal infection. Moreover; the traditional Ayurvedic medicine consider *R. communis* the emperor of therapeutic for curing diseases [11]. The *Ricinus communis* has a strange antimicrobial, insecticidal and regicidal activity and can be used for important therapeutic aid, but it is very toxic, the acute toxicity of fraction isolated form (FRC2A) from the leaves of *Ricinus communis* was determined in male mice hybrid to be (18-20g), the LD50 was calculated using Litchfield and Wilcoxon Method [12]. Therefore; the aim of this study is to observed the specific antibacterial activity of *R. communis* regarding the most common bacterial pathogen.

### Material and Methods

This study was conducted at both Departments of Pharmacology and Microbiology, College of Medicine; Al-Mustansiriyah University in Baghdad, Iraq during June of 2008.

The isolated bacteria were *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (10 isolates per each bacteria). These were isolated from patients by swabbing the infected area (cellulites ; gastroenteritis and urinary tract infection ) by sterile cotton moistened with nutrient broth carried in test tube contained two ml broth [13]. These samples were incubated in nutrient broth at 37 °C for 18 hours, then each sample was sub cultured on blood agar, MaConky agar and incubated at 37°C for 24 hours. The suspected colonies of *Staphylococcus aureus* which were positive for gram stain, catalase , coagulase [14], while the *Enterococcus faecalis*

which were positive for gram stain and negative for catalase. The *Pseudomonas aeruginosa* which were gram negative and oxidase positive give fluorescent yellow-green color at 42 °C. [15] The antibiotic susceptibility test was done by using the antibiotic disc which were applied by using sterile forceps, these antibiotic are Nalidix acid (10 mg), Tetracyclin (10 mg), Amoxicillin (10 mg). The size of zone of inhibition was compared with standard diameter for each antibiotic according to Bauer *et al* method [16]. The plant extraction done by grained the dried seeds of *R.*

*communis* into fine powder, this powder divided into two part one dissolved in distilled water and the second at ethanol 95% for two days, with a ratio of 1g/10ml solvent.

#### **Alcoholic and aqueous Extracts:**

50 gram of seed powder added to 500 ml ethanol 95% and then extracted at 45°C, then concentrating the extract through rotary evaporator at 40°C this represent the alcoholic extract while the aqueous extract prepared through adding 50 gram from seed powder to 500 ml distilled water, then concentrating the extract through rotary evaporator at 40°C then filtered by specific filtered water so the final concentration for both extracts were 10mg/ml. [17,18]

#### **Determination of minimal inhibitory concentration (MIC):**

A double dilution of each extract in Mueller-Hinton broth plates were made, and then each bacteria were inoculated into 40 tubes discretely

and incubated at 37 °C. The cultures were diluted to 100-fold; then alcoholic and aqueous extract of *R. communis* were added at 2mg, 4mg, 8mg, 16mg and 32 mg respectively. The selected antibiotics concentrations were 10 mg which were use as control for alcoholic and aqueous extract of *R. communis* seed.

#### **Results**

The antibiotic sensitivity of the bacterial isolates used in this study showed different susceptibility according to culture and sensitivity for different antibiotics, *P. aeruginosa* showed greater resistance for amoxicillin, while; *E. coli* was sensitive for Nalidixic acid and less sensitive for Amoxicillin. Therefore; the selected bacteria regarded as sensitive for Tetracycline and Nalidixic acid and resistant for Amoxicillin (table 1).

Nalidixic acid has potent effect on *S. aureus* and *E. fecalis* and diminutive effect on *E. coli* and *P. aeruginosa*, while tetracycline generate similar effects to nalidixic acid, but amoxicillin produce smallest effect on all four isolated bacteria. The antibacterial activity of *R. communis* for both aqueous and alcoholic extract of *R. communis* appear in (table 2). The MIC of the aqueous extract of *R. communis* are 16-32 mg/ml for all bacterial species in this study while the MIC for the alcoholic extract of *R. communis* are 8-16 mg/ml for most of the bacterial species in this study. Thus; the MIC for each bacterium regarding the aqueous and alcoholic extract of *R. communis* appeared in (table 4).

**Table 1: Antibiotics sensitivity test.**

antibiotic	<i>S. aureus</i>		<i>E. fecalis</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	S	R	S	R	S	R	S	R
Nalidixic acid	57.4%	42.6%	58.4%	41.6%	33%	67%	33%	67%
Tetracycline	67%	33%	58.4%	41.6%	41%	59%	25%	75%
Amoxicillin	16.6%	83.4%	33%	67%	9%	91%	0%	100%

**Table 2: The MIC of aqueous extract of *R. communis***

Bacteria	2mg/ml	4 mg/ml	8 mg/ml	16 mg/ml	32 mg/ml
<i>E. fecalis</i>	+	+	+	-	-
<i>S. aureus</i>	+	+	+	+	-
<i>P. aeruginosa</i>	+	+	-	-	-
<i>E. coli</i>	+	+	+	-	-

**Table 3: The MIC of alcoholic extract of *R. communis*.**

bacteria	2mg/ml	4 mg/ml	8 mg/ml	16 mg/ml	32 mg/ml
<i>E. fecalis</i>	+	+	-	-	-
<i>S. aureus</i>	+	+	-	-	-
<i>P. aeruginosa</i>	+	+	+	+	-
<i>E. coli</i>	+	+	-	-	-

**Table 4: The MIC of aqueous and alcoholic extract of R.communis.**

Bacteria	MIC	
	aqueous	alcoholic
<i>E. fecalis</i>	16mg/ml	8 mg/ml
<i>S. aureus</i>	32 mg/ml	8 mg/ml
<i>P. aeruginosa</i>	8 mg/ml	16mg/ml
<i>E. coli</i>	16mg/ml	8 mg/ml

## Discussion

The phytochemical analysis of *Ricinus communis* showed the presence of the flavanoid rutin and alkaloid ricinine, these agents might inhibit the mitochondrial respiratory chain [19,20]. Moreover; Perez 2004 reported that flavanoid may be responsible for the antimicrobial activity *R.communis*. [21]

The antimicrobial activity of *R.communis* had been determined previously, some of those authors have reported that this plant has antifungal activity, however; an insignificant effects were determined by Upasani, Kotkar and Mendki et al 2003. [22]

*Ricinus communis* produce significant antibacterial activity against gram negative and less for gram positive bacteria these effects well showed with alcoholic extract, this result supported by Allero study 2006 who showed that successive isolation of botanical compounds from plant stuff are largely dependent on the type of solvent used in the extraction procedure, also the plant extracts prepared with methanol and ethanol provide more reliable antimicrobial activity against resistances bacteria. [23] Furthermore; *Ricinus communis* produces significant antioxidant effects by inhibition of arylhydrocarbon hydroxylase activity and  $H_2O_2$  production by lindan-induced mouse hepatic microsomes, and the methanol extract of *Ricinus communis* demonstrated strong anti-oxidant activity [24]. The results of the present study showed that MIC of alcoholic extract of *Ricinus communis* produces considerable effect more than aqueous extract regarding the gram negative bacteria. To discuss the antimicrobial activity of *Ricinus communis*, we should review the content and phytochemical components of *Ricinus communis*. Ricin is a one of the most thoroughly studies toxin produced by the bean of *Ricinus communis*, many studies have been reported that

the antimicrobial activity of this herb related to the presence of ricin [25]. The ricin is a heterodimeric glycoprotein consisting of A chain and B chain these responsible for cytotoxicity by depurinated the adenine residue of the rRNA, so inactivating the protein synthesis, also the ricin synthesized in the endosperm cell of mature seeds and are stored in an organelle called protein body, when the mature seed germinates, the toxin hydrolyzed within a few day [26]. In addition; lectin is a second main component of *Ricinus communis* act as galactose binding site so interact with the metabolic pathway of bacteria [27]. This plant also produce other toxins that interfere with protein synthesis and that are binary preformed protein [28]. Ricin act through interruption of protein translocation and binding to the glycoprotein and phospholipid receptors on eukaryotic cell membrane that contained a specific residue of galactose; then it internalized by receptor mediated-endocytosis and its catalytic component into cytoplasm where it exert its effects [29]. Moreover; ricin interact with larger subunit of RNA (28s rRNA) and lead to removing the adenine residue by cleavage the N-glycoside bond; but ricin and other inactivating enzyme have a less specific in vitro action on DNA and RNA. [30] Therefore; the *Ricinus communis* produce antimicrobial activity by two mechanisms, throughout inhibition of the protein synthesis and blocking the mitochondrial respiratory chain these per se explain the antimicrobial activity of *Ricinus communis* and its bacteriostatic rather than bactericidal effects due to inhibition of protein synthesis because most antibiotic that act by inhibition of protein synthesis like tetracycline and erythromycin are bacteriostatic. [31] Thus, our study produce lower MIC of *Ricinus communis* for alcoholic and aqueous extract differ from other studies, this may be explained

by the sample size or the type of isolated bacteria which were may be more sensitive to this herb. From this study; we can reach to an important conclusions, that are the Ricinus communis produce antimicrobial activity similar to protein synthesis inhibitor like tetracycline and because it is very toxic regarding the ricin so it should be used topically for infected area. Also we recommend for other study needed to evaluate its effect on fungi and mycobacterium and determine the dose response curve via in vivo study.

### References

1. Alwan A . The role of prosopis farcta in treatment of cutaneous fungal infection. **2000**; (2): 22-5.
2. Mitscer L. A., Lea B ., Wu W .et al. Antimicrobial agents from higher plants. J of Natural products **1972**;3(11): 35-175.
3. Chein W. The medicinal plants in health care .Regional office, Tokyo **1977**;13-17
4. Shamma. A, Mistsche L. A. Comprehensive survery of indigenou results of 327 of potential Economic Value Natural products**1979**;42: 633-42
5. Blumenthal M. et al (editor). The Complet German E Monographs: The therapeutics Guide to Herbal Medicine.American Botonical Council. Integrative Medicine Communication **1998**;(3):5-7
6. Tyler. V. E .What pharmacist should know about herbal remedies.J Am Assoc (Wash).**1999**; **36**(1):29
7. Schulz V. et al .Ration phytotherapy: A physician's Guide to Herbal Medicine-Springer **1998**;2(43):66-7
8. Lennett. E. H .Manual of clinical microbiology 4<sup>th</sup> Ed Washington DC .American Association for Microbiology **1985**;(60): 978-87
9. Alcalá de Marcano, N .Vargas.Efecto de extractors vegetales Thielaviopsis basicota .Rev Fac Agron (LUZ) **2005**; **22**:315-23
10. Zaika L. Spices and herbs: their antimicrobial activity and determination.J food safety (**1975**); 9:97-118
11. Ferraz A. Pharmacological evaluation of ricin.Pharmacolog & Biochemistry and Behavior .**1999**; **63**:367-75
12. Bernard D., Rento D .Microbiology.14<sup>th</sup> ed Philadelphia: Lippincott.**1990**; 540-99
13. Hawkey P. Medical bacteriology a practical approach .NewYork: IRL press **1989**;3(2): 167-93
14. Anderson F., Datta N., and Shaw J.Facer in hospital infection .Bril Med J **1972**;3:382-5
15. Gupta . S. Prospects and perspective of natural plants products in medicine Indian. J of Phar**2001**;26: 1-12
16. Shnerder .B .Quantitaive Determination of Azadirachtin using high liquid chromatography.Nairobi, **1986**;32:10-15
17. Chang S . Resistance to antimicrobial agents.Int.JAntimicrobial Agent **1994**;4:143-46
18. Leonardo M.R, Tanomaru Filho M, Silva L.A, Nelson Filho P, Bonifácio K.C, Ito I.Y. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endodon **1999**;25:167-171.
19. Perez C. Potential medicament contra bacteria's hongos resistance. **2004**;Website <http://www.fcen.uba.ar/prensa/noticias/2004/notiias03may2004-htm/>.
20. Upasani S .M, Kotkar H. M, Mendki P.S et al .Partial characterization and insecticidal properties of R.communis L follage flavanoid.Pest Manaq Sci **2003**; (**59**):1349-54
21. Alero A. Antimicrobial activity of solanum tomentosum .Af.JBrotechnol **2006**; 5;369-72
22. Almedia J. et al .Coma from the health food store.interaction between Kava and alprozolam and methanolic herbal extract.Ann intern Med **1996**; **125**:940-41
23. Knight B. Ricin a potent homicidal poison.Br. Med J **1979**;(278):350-51
24. Robertus J . The stracture and action of ricin, a cytotoxic N-glycosidase.Cell Biol **1991**;(2):23-30
25. Hawkey P.M. Medical bacteriology a practical approach.NewYork.IRL Press **2001**;(44):180-2
26. DerMardosian A. The Lawrence Review of Natural products.Facts and comparison Publishing Group (Serial) .**2007** ;(5):22-4

27. Ferraz A. Pharmacological evaluation of ricin, central nervous system stimulant. *Pharmacology & Biochemistry* **2002;63**:367-754.
28. Siqueira J.R, Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. *J Endodon* **1996;22**:674-676.
29. Hindler J.A, Howard BJ, Keiser JF. Antimicrobial agents and antimicrobial susceptibility testing. In: Howard BJ. *Clinical and Pathogenic Microbiology*. 2nd ed
30. Mantesso A. In vitro evaluation of a new intracanal irrigating solution derived from *Ricinus communis*. *Anais da XVI Reunião Anual da SBPqO* **2000**;1:18.