Antimicrobial properties of 3 medicinal plants from Saudi Arabia against some clinical isolates of bacteria

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ABSTRACT

الأهداف: دراسة تأثير الإيثانول المُستخلص من ثمار نبات السولانيم انكانم، وأوراق الخروع، والكرات وذلك على بعض البكتيريا الممرضة للإنسان، وتقدير المركبات الفينولية فيها.

الطريقة: أُجريت هذه الدراسة في كلية العلوم، جامعة الملك خالد، أبها، المملكة العربية السعودية خلال الفترة من يوليو إلى سبتمبر 2011م. لقد تم استخدام ٩ من العزلات البكتيرية، و3 مستخلصات من الإيثانول لدراسة التأثير الميكروبي. وطُحن 30 غرام من الأجزاء النباتية المختلفة ثم الترشيح و خلط الراشح مع 100 مل إيثانول والرج في الهزاز الدوار لمدة 48 ساعة، وبعد ذلك تم تبخير الإيثانول تماماً من كل عينة ووزنها وتحديد نشاطها البكتيري باستخدام طريقة الانتشار بثقب الآجار. وقد أُستخدم جهاز HPLC لتحديد وتقدير كمية الفينولات المستخلصة في النباتات التي شملتها هذه الدراسة.

النتائج: أظهرت الدراسة أن تلك المستخلصات لها تأثير مضاد على الأنواع البكتيرية الممرضة. وقد أظهر مستخلص الإيثانول من أوراق الخروع أعلى نشاط مضاد للبكتريا، ثم أوراق الكرات، فيما كانت ثمار نبات السولانيم انكانم أقل نشاطاً. وبينت نتائج تحليل الفينولات تواجد كمية أعلى من الفينولات في أوراق نبات الخروع عن تلك التي تتواجد في ثمار نبات السولانيم انكانم أو أوراق نبات الكرات.

خاتمة: أوضحت هذه الدراسة أن مستخلص الإيثانول لتلك النباتات المذكورة في الدراسة قد يكون أحد البدائل للمضادات الحيوية.

Objectives: To examine the *in vitro* antibacterial activity of the ethanol extract of fresh fruits of *Solanum incanum* L., fresh leaves of *Ricinus communis* L. and *Allium ampeloprasum* var. *porrum* L., and to determine and quantify the phenol compounds of the investigated plant parts.

Methods: This study was carried out at the Faculty of Science, King Khalid University, Abha, Kingdom of Saudi Arabia from July 2011 to September 2011. Nine clinical strains of bacteria and 3 ethanol extracts of 3 plant species were used for the antimicrobial study. Thirty grams of each sample was ground, filtrated, and each filtrate mixed with 100 ml ethanol and placed in a shaker for 48 hours. The ethanol was evaporated from the sample, weighed, and subjected to an antibacterial activity test using the agar diffusion method. The high-performance liquid chromatography was used to identify and quantify the phenols extracts of investigated samples.

Results: Ethanol extract of the investigated plant parts showed antibacterial activities against different pathogenic bacteria. Leaf extracts of *Ricinus communis* showed the highest antibacterial activity, followed by the leaves of *Allium ampeloprasum* var. *porrum*, while the fruits of *Solanum incanum* showed the least activity. The amounts of main phenols detected in *Ricinus communis* leaves were higher than those of *Solanum incanum* fruits and *Allium ampeloprasum* var. *porrum* leaves.

Conclusion: The ethanol extract of the tested plants could be considered as an alternative source of new antibacterial drugs.

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Many human diseases are known to have been treated with herbal medicine throughout the history of human beings. The increasing evolution of multi drug resistant bacteria, and the recent appearance of strains with reduced susceptibility to antibiotics leads to the emergence of untreatable bacterial diseases.[1-5] In addition, the revival of interest in plant derived drugs is mainly due to the widespread belief that 'natural medicines' are safe and more dependable than the costly, synthetic drugs, many of which are toxic and

possess adverse effects. Thus, there is a growing interest to explore the alternative drugs from different plant species that have antimicrobial properties and can be used as antibiotic resources.[6,7] In this respect, *Solanum incanum* L. (family: *Solanaceae*), *Ricinus communis* L. (family: *Euphorbiaceae*) and *Allium ampeloprasum* var. *porrum* L. (family: *Alliaceae*) have been used by mankind in the treatment of various diseases. For instance, the information collected from traditional healers indicates that the root extract of *Solanum incanum* can be applied orally for sexually transmitted diseases, and the SR-T100 extracted from it induces cutaneous squamous cell carcinoma apoptosis.[8,9] The methanolic extract of *Ricinus communis* roots exhibited a significant anti-inflammatory, free radical scavenging action, and anti-fertility properties.[10,11] This has led us to screen in vitro antibacterial properties of ethanol extracts of fresh fruits of *Solanum incanum* L., fresh leaves of *Ricinus communis* L., and *Allium ampeloprasum* var. *porrum* L. As there is a strong positive correlation between phenolic compounds and antimicrobial activities,[12-14] some phenols compounds have been investigated to develop new antibiotics drugs with distinguished efficacy and comparable benefits over those already in use.

Methods. ***Collection of plant material.*** Fresh plant materials namely, *Solanum incanum* L. (Fruits), *Ricinus communis* L. (leaves) and *Allium ampeloprasum* var. *porrum* L. (leaves), which were free from disease, were collected from the Aseer Governorate, Kingdom of Saudi Arabia during the period from July 2011 to September 2011. Each plant sample was washed thoroughly several times with distilled water.

Extraction of plant material. Thirty grams of fresh leaves of *Ricinus communis* L., *Allium ampeloprasum* var. *porrum* L. and fresh fruits of *Solanum incanum* L. were weighed out and crushed directly by grinder (Thomas Wiley laboratory mill, model 4 (Thomas Scientific, Swedesboro, New Jersey, USA) screen size one mm) for 15 minutes, and the solution samples were filtered through 2-layered muslin cloth.[15] The filtrates were mixed with 100 ml ethanol, placed in a shaker for 48 hours, and then filtered through Whatman No. 1 filter paper (Madhu Chemical Company, Maharashtra, India). The ethanol in each filtrate was evaporated completely, and each extract was weighed and subjected to an antibacterial activity test.

Preparation of human pathogenic bacterial cultures. Nine clinical strains of bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* sp., *Proteus* sp., *Klebsiella pneumoniae*, *Micrococcus* sp., *Staphylococcus epidermidis* and *Bacillus subtilis*) were obtained from the Microbiology Laboratory, Faculty of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia. All the test strains were first subcultured in nutrient broth at 37°C for 24 hours and used as test pathogens.

Antimicrobial activity. The antimicrobial activity was determined by the agar well diffusion method against different strains of bacteria.[16] 100 µl of standardized inoculum of each test bacterium was spread onto sterile Muller-Hinton Agar (Hi-Media). A 6 mm diameter well was cut from the agar using a sterile cork-borer, subsequently each well was filled with 0.1 ml of the ethanol plant extraction. Sterile dimethyl sulfoxide (DMSO) served as negative, and gentamicin (10 ug/disc) as positive controls to determine the sensitivity of each bacterial species tested. The plates were kept at room temperature for one hour to allow proper diffusion of the extract into agar and then incubated at 37°C for 24 hours. Nine clinical strains of bacteria, and 3 ethanol extracts of 3 plant species were used for the antimicrobial study. The tests were performed in triplicate, and the minimal inhibitory concentration (MIC) and diameter of the cleared zones were determined. All tests were carried out from July 2011 to September 2011 at the Biology Department, College of Science, King Khalid University, Kingdom of Saudi Arabia, and the study was approved by the Faculty Ethics Committee.

Quantitation of phenols using high-performance liquid chromatography (HPLC) apparatus. A Shimadzu model HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a solvent delivery module (LC-10AD) with a double plunger reciprocating pump, UV-VIS detector (SPA-10A), column oven (CTO-10A) and 20-ul injection loop were used. The column used was an Apex octadecyl 104 C_{18} (25x0.4 cm ID) with 5-um packing (Jones Chromatography Limited, Colorado, USA).[17] Phenolic compounds present in the samples were identified by comparing retention time (Rt) of the standards and by the co-injection. Contents of phenolic compounds were calculated by comparing peak areas of reference compound with those in the samples run under similar elution conditions.[18]

Statistical analysis. The results of the antimicrobial activity were expressed as the means obtained from 3 independent analyses. Analysis of variance was used to compare between data. All analyses were performed at $p<0.05$ using Minitab version 2000 13.1 (Minitab, State College, PA, USA).

Results. ***Antibacterial screening.*** The results of the antimicrobial activity of the ethanol extract of fresh fruits of *Solanum incanum* L., fresh leaves of *Ricinus communis*

L. and *Allium ampeloprasum* var. *porrum* L. by agar well diffusion method revealed that all 3 extracts showed inhibitory activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* sp., *Proteus* sp., *Klebsiella pneumoniae*, *Micrococcus* sp., *Staphylococcus epidermidis* and *Bacillus subtilis* as shown in **Table 1** and **Figure 1**. Among the 3 plants tested, the ethanol leaf extracts of *Ricinus communis* showed the highest antibacterial activity with a zone of inhibition ranging from 17.46 - 27.33 mm at 20 mg/ml concentration and MIC value at 10 mg/ml. It showed potent antibacterial activity against *Micrococcus* sp, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* sp., *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *Proteus* sp., while being less active against *Bacillus subtilis* and *Pseudomonas aeruginosa*. The ethanolic extract of the leaves of *Allium ampeloprasum* var. *porrum* exhibited moderate antibacterial activity with respective means between 13.33 - 23mm inhibition zone at 23 mg/ml concentration and MIC value of 11.5 mg/ml. It showed highest inhibitory activity against *Pseudomonas aeruginosa* and *Micrococcus* sp., and moderate activity against *Escherichia coli*, *Acinetobacter* sp., *Proteus* sp. and *Klebsiella pneumoniae* and least activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*. On the other hand, the ethanol extract of the fresh fruits of *Solanum incanum* have a slightly lower antibacterial activity with a zone of inhibition ranging from 8.46 to 22.33 mm at 25 mg/ml concentration and MIC value of 12.5 mg/ml. It was found to be active against *Micrococcus* sp., *Staphylococcus epidermidis* and *Staphylococcus aureus* and less active against *Acinetobacter* sp., *Proteus* sp., *Klebsiella pneumonia*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, while it exhibited much less activity against *Escherichia coli*. Negative control wells did not show any lack of bacterial growth, while the gentamicin discs used as a positive control showed halo indicative lack of bacterial growth in all plates.

Phenols content. The HPLC analysis of the ethanol extract of fresh fruits of *Solanum incanum* L. and fresh leaves of *Ricinus communis* L. and *Allium ampeloprasum* var. *porrum* L. allowed the identification of 5 phenolic compounds: Catechin, *p*-coumaric acid, ferulic acid, cinnamic acid and sinapic acid. From the results shown in **Table 2**, it can be stated that the type and amount of phenolic compounds detected varied in terms of the investigated plants. The results showed also that the catechin of *Ricinus communis* leaves was significantly higher than those of *Solanum incanum* fruits and *Allium ampeloprasum* var. *porrum* leaves. P-coumaric acid, ferulic acid, and cinnamic acid levels of *Ricinus communis* leaves were higher than those of *Allium ampeloprasum* var. *porrum* leaves, but these compounds were not traced in fruits of *Solanum incanum*. In addition, the amount of sinapic acid in *Solanum incanum* fruits was slightly higher than in the leaves of *Ricinus communis*, while it could not be detected in the *Allium ampeloprasum* var. *porrum* leaves.

Table 1 - Antibacterial activity and MIC values of ethanol extract of fresh leaves of *Ricinus communis* L., *Allium ampeloprasum* var. *porrum* L. and fresh fruits of *Solanum incanum* L. Values are expressed as mean of the 3 replicates.

Bacterial sp.	*Solanum incanum* (Fresh fruits)		*Ricinus communis* (Fresh leaves)		*Allium ampeloprasum var. porrum* (Fresh leaves)		DEMSO	Gentamicin 95% CI
	(MIC)				(MIC)			
	25mg/ml	12.5 mg/ml	20 mg/ml	10 mg/ml	23 mg/ml	11.5 mg/ml		10 ug/disc
	(95% CI)							(95% CI)
Pseudomonas aeruginosa	11.33 (10.62-11.97)	7.43 (6.43-8.42)	17.53 (14.15-20.90)	9.00 (6.78-11.21)	23.00 (18.57-27.42)	13.33 (9.93-16.72)	NI	31.33 (31.15-31.50)
Staphylococcus aureus	18.00 (15.78-20.21)	7.66 (6.39-8.92)	25.60 (22.24-28.95)	16.36 (14.34-18.37)	13.33 (9.08-17.57)	10.36 (7.62-13.09)	NI	29.33 (29.15-29.50)
Escherichia coli	8.46 (6.67-10.24)	7.56 (6.47-8.65)	25.60 (24.43-26.76)	17.46 (16.34-18.57)	17.16 (13.70-20.61)	13.00 (10.31-15.68)	NI	32.33 (32.25-32.40)
Acinetobacter sp.	10.23 (8.71-11.74)	7.36 (6.47-8.26)	23.26 (21.84-24.67)	16.46 (14.67-18.24)	15.36 (14.46-16.25)	10.33 (9.06-11.59)	NI	23.77 (23.72-23.81)
Proteus sp.	13.00 (8.57-17.42)	11.26 (8.87-13.64)	19.33 (14.70-23.95)	17.10 (14.91-19.28)	17.26 (16.26-18.25)	7.60 (6.43-8.76)	NI	23.33 (23.15-23.50)
Klebsiella pneumoniae	13.00 (10.44-15.55)	10.66 (8.10-13.21)	20.60 (15.48-25.71)	13.70 (11.21-16.18)	15.26 (11.83-18.68)	7.26 (6.24-8.27)	NI	36.67 (36.52-36.81)
Micrococcus sp.	22.33 (17.70-26.95)	7.60 (6.35-8.84)	27.33 (20.92-33.73)	13.36 (12.14-14.57)	20.00 (18.68-21.31)	17.66 (15.10-20.21)	NI	31.67 (31.52-31.81)
Staphylococcus epidermidis	19.20 (16.06-22.33)	9.16 (5.26-13.06)	20.66 (18.10-23.21)	15.36 (12.08-18.63)	14.00 (10.89-17.10)	7.63 (5.91-9.34)	NI	31.67 (31.52-31.81)
Bacillus subtilis	12.16 (10.47-13.84)	7.83 (5.52-10.14)	17.46 (16.34-18.57)	13.86 (11.59-16.12)	13.40 (8.60-18.19)	9.20 (6.86-11.53)	NI	31.00 (30.97-31.02)

NI - no inhibition zone, DEMSO - dimethyl sulfoxide, MIC - minimum inhibitory concentration, CI - confidence interval at $p<0.05$

Discussion. Medicinal plants play a crucial role in the search for alternative antimicrobial components. According to the World Health Organization, it is estimated that around 80% of the earth's population use some form of herbal medicine in their health care, whereas natural products are a preferable option than synthetic ones.[19] The literature indicates that medicinal plants have secondary compounds that are of great importance in human life in terms of acting as antioxidants, anti-inflammatories, and being involved in the modulation of detoxification enzymes, the stimulation of the immune system, the modulation of steroid metabolism and antimicrobial effects.[20] The results obtained in the present study indicate that the ethanol extract of fresh fruits of *Solanum incanum* and the fresh leaves of *Ricinus communis* and *Allium ampeloprasum* var. *porrum* have varied antimicrobial activities to the test organisms used, namely *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter* sp., *Proteus sp*., *Klebsiella pneumoniae*, *Micrococcus* sp., *Staphylococcus epidermidis* and *Bacillus subtilis*. The results also show that different plants assayed here possess different levels of antimicrobial activities, that ethanol extracts of fresh leaves of *Ricinus communis* exhibited the highest activity, followed by the leaves of *Allium ampeloprasum* var. *porrum*, while the fruits of *Solanum incanum* demonstrated the least activity. The findings support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds.[21-23] Moreover, the currents results support findings of other researchers who found the presence of antimicrobial activity in essential oil of *Allium ampeloprasum*, in oil paste of *Ricinus communis,* and in aqueous and methanol extracts of the leaves of *Solanum incanum*.[24-26] It was demonstrated that the pharmacological activity derived from crude leaves and pericarp aqueous extracts of *Ricinus communis* may be due to the presence of tannins, steroids and flavonoids.[27] A number of biologically active compounds such as organosulphurous compounds like alliin and aliicin have been identified in *Allium* species which possess antibacterial, antifungal, antiviral, antitumour, anticoagulation, antihypertensive, antiparasitic, and hepatoprotective activity.[28-31] Also the extract of *Solanum incanum* showed the presence of saponins that have healing properties as a natural blood cleanser, expectorant, and antibiotic.[32] However, the literature contains scant information regarding the relationship between the phenolic content and the antimicrobial activity of these 3 plants.

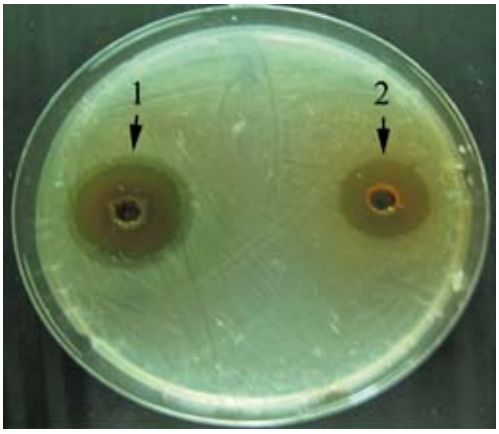

Figure 1 - Inhibition zone of *Bacillus subtilis* caused by ethanol extract of fresh leaves of *Ricinus communis* (1) and *Allium ampeloprasum* var. *porrum* (2) as indicated by arrows.

Table 2 - Contents of 5 phenols (catechin, p-coumaric acid, ferulic acid, cinnamic acid, sinapic acid) in the ethanol extract of fresh leaves of *Ricinus communis* L., *Allium ampeloprasum* var. *porrum* L. and fresh fruits of *Solanum incanum* L.

Analyte	*Solanum incanum L.* Fresh fruits		*Ricinus communis L.* Fresh leaves		*Allium ampeloprasum var. porrum L.* Fresh leaves	
	RT (min)	mg/100g	RT (min)	mg/100g (95% CI)	RT (min)	mg/100g
Catechin	2.095	11.81 (8.76-14.19)	2.086	20.18 (13.37-27.22)	2.125	7.40 (3.06-12.91)
P-coumaric acid	ND	ND	12.97	0.019 (0.001-0.04)	12.33	Tr
Ferulic acid	ND	ND	13.62	0.026 (0.003-0.05)	13.66	Tr
Cinnamic acid	ND	ND	14.12	0.008 (0.001-0.017)	13.97	Tr
Sinapic acid	19.96	0.023	20.00	0.020 (0.005-0.05)	ND	ND

RT - retention time of the peak, Tr - concentration <0.001 mg/100 g, ND - not detected, min - minutes, CI - confidence interval at $p<0.05$

In this study, most of the antimicrobial activity in ethanol extracts of investigated plants appears to be explainable by phenolic compounds including catechin, *p*-coumaric acid, ferulic acid, cinnamic acid, and sinapic acid. The contents of the main phenols in *Ricinus communis* were higher than those of *Solanum incanum* and *Allium ampeloprasum* var. *porrum*. These bioactive components that are naturally occurring in most plant materials have been reported to account for the exertion of antimicrobial activity.[33-38] The results highlight the strong positive relationship between antimicrobial activity and the phenol content in all the plant extracts examined. These results are in agreement with data previously reported in the literature.[39-41] From a clinical point of view, *Pseudomonas aeruginosa* is one of the most common microbes to cause nosocomial infections that mainly affect immuno-compromised patients with dangerous underlying diseases.[42] *Staphylococcus aureus* is the main cause of bacterial infections in many countries affecting the bloodstream, the lower respiratory tract, skin, and soft tissue.[43] Infection caused by *Escherichia coli* and *Klebsiella pneumoniae* are among the most important causes of severe nosocomial and community-associated bacterial infections in human beings, and the resistance of these bacteria to antimicrobial medicine is a serious concern.[44] *Acinetobacter* sp is resistant to many antibiotics, meaning that medical treatment is complicated.[45] *Proteus* is considered as a renal pathogen especially in causing upper UTI because of its propensity to promote struvite renal calculi and possesses various virulence factors that enhance its urinary epithelial invasiveness rendering this microbe resistant to antibiotics.[46,47] *Staphylococcus epidermidis* has been considered as a common nosocomial pathogen affecting immuno-compromised persons carrying medical instruments.[48] *Micrococcus* species is a common cause of bloodstream infections among patients subjected to long-term IV epoprostenol.[49] The fact that the ethanol extract of the plants studied were active against both clinical and laboratory isolates, is an indication that it can be used as an important source of very potent antibiotic drugs. Investigating the toxic effects of ethanol extracts of these plants could be a limitation of this study.

In conclusion, this study has shown that ethanol extracts from the fresh leaves of *A. ampeloprasum* var. *porrum*, *Ricinus communis,* and fresh fruit of *Solanum incanum* possess antimicrobial properties. It can be suggested that ethanol extracts of these plants are a great potential source of antibacterial compounds that could be used in the formulation of new antimicrobial drugs of natural basis. The bioactive phenolic substances obtained from these plants can therefore be a promising antibiotic source for the treatment of various bacterial infections. Isolation, characterization, and purification of these phytoconstituents, and the determination of their respective antibacterial activities, together with a toxicological analysis should be the future direction for researchers.

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