



Sudan University of Science and Technology  
Collage of Graduate Studies



***Invitro* Assessment of Antimicrobial Activity of Methanolic  
and Aqueous Extracts of *Zingiber officinale* Roots  
and *Citruslimon* Peels against Multi-drug Resistant Urinary  
Tract Infections Isolates**

التقييم المعملّي للنشاط المضاد للميكروبات لمستخلصات جذور الزنجبيل وقشور الليمون ضد  
البكتريا المقاومة للأدوية المتعددة المعزولة من عينات مرضى التهابات المسالك البولية

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# الآية

قال تعالى:

﴿ إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ۗ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ ﴾

صدق الله العظيم

سورة فاطر الآية (28)

# **DEDICATION**

To

My father

My mother

My wife

My brothers

My friends

## ACKNOWLEDGMENTS

First of all, all praises to Almighty *ALLAH*, who gave me the strength and patient to finish this work. And all thanks to my supervisor **Dr. Wafa Mohammed Abdalla** for her guidance, advice and patience during this study. I give special thanks to my colleague **Esraa Abdelrhman Abdalla** for her continued support and guidance without her I was not able to accomplish this work. Deep thanks to Sudan University of science and technology –Microbiology lab staff and University of Health and Medical Services Hospital-University of Khartoum for their help to continue this work.

## ABSTRACT

Herbal medicines have been widely used all over the world since ancient times and have been recognized by physicians and patients for their better therapeutic value as they have fewer adverse effects as compared with modern medicines. This is experimental study aimed to determine the antimicrobial activity of water and methanolic extracts of *Zingiber officinale* roots and *Citrus limon* peels using agar disk-diffusion method. The study was conducted in Khartoum state, Sudan, during February to October 2019. Hundred clinical isolates from urine specimens were collected from different hospitals. The clinical isolates were subcultured in Cysteine Lactose Electrolyte Deficient (CLED) agar and then reidentified using Gram's stain and biochemical reactions. The plant extractions was carried out using Soxhlet extraction method for methanolic extracts and maceration (cold) extraction method for water extracts of both plants. The identified species were 79(79%) Gram's negative bacilli include: *Escherichia coli* 37 (37%), followed by *Proteus vulgaris* 21 (21%), *Klebsiella pneumoniae* 13(13%), *Pseudomonas aeruginosa* 8 (8%). While Gram's positive cocci were 21 (21%) isolates, they are *Enterococcus faecalis* 18(18%) and 3 *Staphylococcus aureus* isolates (3%). Susceptibility testing was performed using standard and clinical isolates against number of antibiotics, the result showed that the highest percentage of resistant was against Co-Amoxiclav (69%) followed by Nalidixic acid (50%). Only the isolated bacteria which was multidrug resistant, they were tested for their sensitivity to *Zingiber officinale* and *Citrus limon*. They were distributed as follow: *Escherichia coli* 18/37 (48.6%), *Proteus vulgaris* were 12/21 (57.1%), *Klebsiella pneumoniae* 8/13 (61.5%) and *Pseudomonas aeruginosa* 8/8 (100%). The study concluded that, the aqueous and methanolic extracts of *Zingiber officinale* showed activity against multidrug resistant *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. While the methanolic and water extract of *Citrus limon* showed activity against multidrug resistant *proteus vulgaris*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) of both methanolic and water extracts of *Zingiber officinale* was 50 mg/ml for *Escherichia coli*, 3.125mg/ml for *Pseudomonas*

*aeruginosa*, while *Klebsiella pneumonia* was (25 and 50) mg/ml for methanolic and water respectively. The MIC for both methanolic and water extracts of *Citrus limon* was 3.125mg/ml for all tested isolates.

## مستخلص الأطروحة

أستخدم طب الأعشاب في جميع أنحاء العالم بصورة واسعة منذ العصور القديمة، وعرف لدى الأطباء والمرضى بقيمته العلاجية العالية وقلة آثاره الجانبية مقارنة بالطب الحديث. الهدف من هذه الدراسة كان اختبار فعالية مستخلصي الماء والميثانول لنباتي الزنجبيل (الجزور) والليمون (القشور) باستخدام طريقة انتشار القرص. أجريت هذه الدراسة في ولاية الخرطوم في السودان في الفترة من فبراير إلى أكتوبر 2019. تم جمع مئة بكتريا معزولة من عينات البول من عدد من المستشفيات. العينات المعزولة تم إعادة تزييعها في سيستين الاكتوز ناقصة الشحنت ثم أعيد التعرف عليها باستخدام صبغة الجرام والتفاعلات الكيموحيوية. تم إجراء عملية استخلاص النباتين بطريقة السوكسيليت للمستخلص الميثانولي وطريقة النقع البارد للمستخلص المائي لكلا النباتين. الأنواع التي تم التعرف عليها كانت 79 بكتريا عصوية سالبة الجرام (79%)، تضمنت الاشريكية القولونية 37 (37%) تتبعها المتقلبة الاعتيادية 21 (21%)، ثم الكلبسيلا الرئوية 13 (13%)، تليها الزائفة الزنجارية 8 (8%)، و21 بكتريا موجبة الجرام، تضمنت المكورة المعوية البرازية 18 (18%) تليها المكورة العنقودية الذهبية 3 (3%). تم اختبار حساسية المضادات الحيوية باستخدام طريقة الانتشار الطبقي القياسي ضد البكتريا القياسية والمعزولة من العينات الطبية وأظهرت النتائج أن أعلى نسبة مقاومه كانت ضد كواموكسكلاف (69%) ثم حمض الناليدكسك (50%). فقط البكتريا المقاومة متعددة الأدوية، تم اختبار حساسيتها ضد مستخلصات الزنجبيل والليمون، والتي تم توزيعها كالاتي: الاشريكية القولونية 18\37 (48.6%)، المتقلبة الاعتيادية 12\21 (57.1%)، الكلبسيلا الرئوية 8\13 (61.5%) والزائفة الزنجارية 8\8 (100%). خلصت الدراسة إلى أن المستخلصين المائي والميثانولي اظهرا فعالية ضد البكتريا المقاومة الاشريكية القولونية، الكلبسيلا الرئوية والزائفة الزنجارية. بينما المستخلصين المائي والميثانولي لليمون اظهر فعالية ضد المتقلبة الاعتيادية، الاشريكية القولونية، الكلبسيلا الرئوية والزائفة الزنجارية. أظهرت الدراسات أن التركيز المثبط الأدنى لمستخلصي الميثانولي والمائي للزنجبيل ضد الاشريكية القولونية (50مج/مل)، والزائفة الزنجارية (3.125 مج/مل)، بينما الكلبسيلا الرئوية (25، 50 مج/مل) للمستخلصين علي التوالي. بينما المستخلصين الميثانولي والمائي لليمون كان (3.125 مج/مل) لكل أنواع البكتريا المعزولة.

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## List of Abbreviations

<b>NO</b>	<b>Abbreviation</b>	<b>Definition of Abbreviation</b>
1	UTIs:	<b>Urinary Tract Infections.</b>
2	WHO:	<b>World Health Organization.</b>
3	MIC:	<b>Minimum Inhibitory Concentration.</b>
4	GPIU:	<b>Global Prevalence Infections in Urology.</b>
5	HAI:	<b>Health-care Associated Infections.</b>
6	VP:	<b>Vogues Proskar test.</b>
7	MR:	<b>Methyle Red test.</b>
8	MDR:	<b>Multi-Drug Resistant Pathogens.</b>
9	CLED:	<b>Cysteine Lactose Electrolytes Deficient medium.</b>
10	KIA:	<b>Kligler Iron Agar test.</b>
11	DNAase:	<b>Deoxyribo Nuclease test.</b>
12	DMSO:	<b>Di Methyle Sulfa Oxide.</b>
13	ATCC:	<b>American Type Classification Committee.</b>
14	SPSS:	<b>Statistical Packages for Social Sciences.</b>