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In vitro antimicrobial activities of two medicinal plant extracts against selected urinary pathogens and their synergistic effects with synthetic antibiotics

*[1,2]Baba, R. T., [1]Oluboyo, B. O., [1]Egbebi, A. H., [2]Dangana, Z. A., [2]Muhammad, Z. K., [2]Sowole, H. B., [2]Sani, H. U., [2]Yakubu, F., [3]Aladodo, M., [2]Babatunde, S. K., and [4]Muhammed, T. O.

[1]Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[2]Department of Medical Laboratory Science, Faculty of Health Sciences, Al-Hikmah University, Ilorin, Nigeria
[3]Department of Immunology, Faculty of Nursing and Allied Health Sciences, University of Abuja, Nigeria
[4]Department of Medical Laboratory Science, Faculty of Health Sciences, Elizade University, Ilara-Mokin, Ondo State, Nigeria
*Correspondence to: omotayo.rasheedat@gmail.com; rtbaba@alhikmah.edu.ng; +2347065391534; +2349026144501

Abstract:

Background: Antibiotic resistance is currently a major global health concern. Multidrug-resistant (MDR) bacteria are emerging and spreading rapidly, threatening the ability to treat common infectious diseases such as urinary tract infection (UTI). The objective of this study is to determine the *in vitro* antimicrobial activities of two medicinal plants (*Euphorbia hirta* and *Senna alata*) and their synergistic effects with synthetic antibiotics.
Methodology: Aqueous and methanol extracts of the two plants were done using Soxhlet and cold maceration techniques. The antimicrobial activities of the extracts were assessed against selected clinical microbial isolates (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans*) using the agar well diffusion while Kirby-Bauer disc diffusion was used for antimicrobial susceptibility testing of the isolates to some selected synthetic antibiotics as comparator. The minimum inhibitory concentration (MIC) of the extracts against the isolates was determined by the broth dilution method, and the minimum bactericidal concentration (MBC) was determined by sub-culturing samples from MIC tubes with no visible growth onto plain agar to determine the lowest concentration that resulted in 99.9% microbial kill. Synergy of synthetic anti-microbials and the plant extracts was determined by the modified disk diffusion test. Data were analyzed using SPSS version 25.0. The Student's *t*-test was used to compare the mean zone diameter of inhibition (ZDI) of triplicate tests for each of the extract on the isolates, and $p<0.05$ was considered statistically significant.
Results: For *E. hirta*, methanol extracts exhibited significantly higher inhibitory activity ($p<0.05$) than aqueous extracts, particularly against *P. aeruginosa* (mean ZDI: 20.3±0.577mm vs 7.7±0.577mm) and *C. albicans* (mean ZDI: 20.7±1.155mm vs 6.0±0.577mm), with MIC values of 6.25mg/ml and 25.0mg/ml respectively. Similarly, for *S. alata*, methanol extracts exhibited higher inhibitory activity than aqueous extracts especially against *S. aureus* (mean ZDI: 21.3±1.528mm vs 6.0±1.155mm), but low inhibitory activity was observed against *K. pneumoniae* (mean ZDI: 7.0±1.000mm vs 6.0±0.577mm). Synergy testing showed enhanced antimicrobial activities when aqueous and methanol extracts were combined with synthetic antibiotics especially imipenem, producing significantly larger mean ZDI against *S. aureus* (45.6±0.577mm vs 48.3±0.577mm for *S. alata* and 38.3 ± 1.15mm vs 41.3±1.15mm for *E. hirta*). The combined extracts of the two plants also demonstrated significantly higher mean ZDI for methanol compared to aqueous extracts against *E. coli* (24.0±1.00mm vs 6.3±0.577mm), *K. pneumoniae* (20.3±0.577mm vs 6.7±1.155mm) and *C. albicans* (21.0±1.155mm vs 6.3±0.577mm).
Conclusion: The synergistic interactions between plant extracts and synthetic antibiotics in this study suggests a potential application in treatment of infections caused by antibiotic-resistant pathogens.

Keywords: *Euphorbia hirta, Senna alata*, Antibiotics, Antimicrobial activities, Synergistic.

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Activités antimicrobiennes *in vitro* de deux extraits de plantes médicinales contre certains pathogènes urinaires et leurs effets synergétiques avec des antibiotiques de synthèse

*[1,2]Baba, R. T., [1]Oluboyo, B. O., [1]Egbebi, A. H., [2]Dangana, Z. A., [2]Muhammad, Z. K., [2]Sowole, H. B., [2]Sani, H. U., [2]Yakubu, F., [3]Aladodo, M., [2]Babatunde, S. K., et [4]Muhammed, T. O.

[1]Département de Sciences de Laboratoire Médical, Faculté de Médecine et des Sciences de la Santé, Université Afe Babalola, Ado-Ekiti, Nigéria
[2]Département de Sciences de Laboratoire Médical, Faculté des Sciences de la Santé, Université Al-Hikmah, Ilorin, Nigéria
[3]Département d'Immunologie, Faculté des Sciences Infirmières et des Sciences Paramédicales, Université d'Abuja, Nigéria
[4]Département de Sciences de Laboratoire Médical, Faculté des Sciences de la Santé, Université Elizade, Ilara-Mokin, État d'Ondo, Nigéria
*Correspondance à: omotayo.rasheedat@gmail.com; rtbaba@alhikmah.edu.ng; +2347065391534; +2349026144501

Résumé:

Contexte: La résistance aux antibiotiques est actuellement un problème de santé mondial majeur. Les bactéries multirésistantes (MDR) émergent et se propagent rapidement, menaçant la capacité à traiter des maladies infectieuses courantes telles que les infections urinaires (IU). L'objectif de cette étude est de déterminer les activités antimicrobiennes in vitro de deux plantes médicinales (*Euphorbia hirta* et *Senna alata*) et leurs effets synergétiques avec les antibiotiques de synthèse.
Méthodologie: Des extraits aqueux et méthanoliques des deux plantes ont été réalisés par Soxhlet et macération à froid. Français Les activités antimicrobiennes des extraits ont été évaluées contre des isolats microbiens cliniques sélectionnés (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* et *Candida albicans*) en utilis333ant la diffusion en puits d'agar tandis que la diffusion sur disque de Kirby-Bauer a été utilisée pour les tests de sensibilité antimicrobienne des isolats à certains antibiotiques synthétiques sélectionnés comme comparateur. La concentration minimale inhibitrice (CMI) des extraits contre les isolats a été déterminée par la méthode de dilution en bouillon, et la concentration minimale bactéricide (CMB) a été déterminée par sous-culture d'échantillons de tubes CMI sans croissance visible sur de la gélose ordinaire pour déterminer la concentration la plus faible qui a entraîné une élimination microbienne de 99,9 %. La synergie des antimicrobiens synthétiques et des extraits de plantes a été déterminée par le test de diffusion sur disque modifié. Les données ont été analysées à l'aide de SPSS version 25.0. Français Le test *t* de Student a été utilisé pour comparer les diamètres moyens de la zone inhibitrice des tests en triple pour chacun des extraits sur les isolats, et $p<0,05$ a été considéré comme statistiquement significatif.
Résultats: Pour *E. hirta*, les extraits au méthanol ont montré une activité inhibitrice significativement plus élevée ($p<0,05$) que les extraits aqueux, en particulier contre *P. aeruginosa* (ZDI moyen: 20,3±0,577mm contre 7,7±0,577mm) et *C. albicans* (ZDI moyen: 20,7±1,155mm contre 6,0±0,577mm), avec des valeurs de CMI de 6,25mg/ml et 25,0mg/ml respectivement. Français De même, pour *S. alata*, les extraits au méthanol ont montré une activité inhibitrice plus élevée que les extraits aqueux, en particulier contre *S. aureus* (ZDI moyen: 21,3±1,528mm contre 6,0±1,155 mm), mais une faible activité inhibitrice a été observée contre *K. pneumoniae* (ZDI moyen: 7,0±1,000mm contre 6,0±0,577mm). Les tests de synergie ont montré des activités antimicrobiennes améliorées lorsque les extraits aqueux et au méthanol étaient combinés avec des antibiotiques synthétiques, en particulier l'imipénème, produisant un ZDI moyen significativement plus élevé contre *S. aureus* (45,6±0,577mm contre 48,3±0,577mm pour *S. alata* et 38,3±1,15mm contre 41,3±1,15mm pour *E. hirta*). Les extraits combinés des deux plantes ont également montré un ZDI moyen significativement plus élevé pour le méthanol par rapport aux extraits aqueux contre *E. coli* (24,0±1,00 mm contre 6,3±0,577mm), *K. pneumoniae* (20,3±0,577mm contre 6,7±1,155mm) et *C. albicans* (21,0±1,155mm contre 6,3±0,577mm).
Conclusion: Les interactions synergétiques entre les extraits de plantes et les antibiotiques de synthèse dans cette étude suggèrent une application potentielle dans le traitement des infections causées par des agents pathogènes résistants aux antibiotiques.

Mots-clés: *Euphorbia hirta, Senna alata*, Antibiotiques, Activités antimicrobiennes, Synergique.

Introduction:

Antibiotic resistance is currently a major global health concern. Multidrug-resistant (MDR) bacteria are emerging and spreading rapidly, threatening the ability to treat common infectious diseases such as urinary tract infection (UTI) (1). UTI represents one of the most common infectious diseases encountered in medical practice today affecting all age groups from neonates to geriatrics (1). It is estimated that about 35% of healthy women suffer symptoms of UTI at some stages in their life. About 5% of women each year suffer from painful and frequent urination (2). The incidence of UTI is greater in women compared to men which may be either due to anatomical predisposition or urothelial mucosal adherence to the mucopolysaccharide lining or other host factors (3).

The extended spectrum β-lactamase (ESBL)-producing bacteria identified among the members of the family Enterobacteriaceae, are increasingly causing UTI both in hospitalized patients and outpatients (4,5). The increasing drug resistance among these bacteria has made therapy of UTI difficult. Indiscriminate use of antibiotics is considered to be one

of the main reasons for emergence of drug-resistance. According to the World Health Organization (WHO), drug-resistant infections could cause 10 million deaths each year by 2050 and significant damage to the global economy (6). Thus, it is very crucial to identify products with antimicrobial properties that could be utilized to develop novel and efficient antibiotics.

Plant products have been used since ancient times to treat diseases and the practice is continued till today. It is estimated that about 80% of the population from developing countries uses medicines derived from plant species (7). As plant-derived medicines are safer than synthetic alternatives (8), their usage will continue to grow in the future. It is expected that by the year 2050, the value of international trade of medicinal plants and their products would reach 5 trillion USD (9). Plants produce a variety of compounds that are not used in their primary metabolism but increase their ability to survive environmental challenges. These secondary metabolites such as alkaloids, phenols, terpenes and others are bioactive and provide defense against herbivores and microbes (10). Different parts of plants such as the leaf, root, flower, fruit, peel, bark, seed, stem, rhizome, etc can be used for therapeutic purposes as they contain high amounts of bioactive compounds with therapeutic potential (11).

Nigeria is a tropical country with a rich variety of medicinal plants. It is estimated that there are several plant species which are expected to have therapeutic benefits (12). Over the last decade, numerous studies have been conducted to analyze the antibacterial properties of eclectic plants. However, a more diverse range of medicinal plants needs to be analyzed extensively to further enhance knowledge. Thus, this study is designed to evaluate the antimicrobial properties of some plants in Nigeria that are used as herbal medicine treatment of many diseases, including *Senna alata* and *Euphorbia hirta.*

Senna alata (also known as ringworm bush or *Cassia alata*) and *E. hirta* (commonly known as 'Tawa-Tawa') have attracted attention due to their wide range of biological activities. Both plants are traditionally used in folk medicine for various ailments, and recent studies have suggested that their extracts may exhibit potent antimicrobial effects, particularly against UTI-causing pathogens (13). In this study, *in vitro* antimicrobial activities of the two medicinal plants and their synergistic eff with synthetic antibiotics were determined.

Materials and method:

Study area and design:

The study was an evaluation of the *in vitro* antimicrobial activities of two medicinal plants (*S. alata* and *E. hirta*) on selected urinary pathogens and their synergistic effects with selected synthetic antibiotics carried out in Medical Microbiology and Parasitology Laboratory of Al-Hikmah University, Ilorin, and University of Ilorin Teaching Hospital, Ilorin, Nigeria.

Ethical clearance for the study:

Ethical approval with reference number (UITH/CAT/189/21^{B}/626) was obtained from University of Ilorin, Teaching Hospital, Nigeria prior to the commencement of the study.

Plant collection, authentication and pulverization of the plant leaves:

Fresh leaves of *E. hirta and S. alata* were collected from the premises of Kwara State stadium Ilorin, Nigeria. The plants were identified and authenticated at the herbarium unit of the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, where they were given herbarium voucher number UILH/001/1511/2024 and UILH/001/1059/ 2024 respectively. The leaves were washed thoroughly separately and air dried in shade for two weeks. Dried leaves were ground into a coarse powder using an industrial blender.

Preparation of the plant extract:

Methanol and aqueous extracts of *E. hirta* and *S. alata* leaves were prepared using Soxhlet extraction and maceration methods respectively (14). For the methanol extracts, 100g of powdered leaves were placed in the Soxhlet apparatus thimbles and extracted with 500 ml of methanol through continuous solvent reflux until exhaustion. The resulting extracts were concentrated using a rotary evaporator at 50°C and then stored at 4°C.

For the aqueous extraction, 100g of ground leaves were soaked in 200 ml distilled water for 72 hours at room temperature with occasional stirring. After filtration, the extracts were frozen at -20 °C and lyophilized at -50°C under vacuum to obtain dry powders. The dried powder was stored at 4°C for further microbiological and phytochemical analyses. The percentage yield of the plant extract was calculated using the formula; Weight of the extract (g) / Weight of the plant × 100 (15).

Preparation of extract stock solution:

The extract stock solution was prepared by dissolving 2g of each dry plant extract in 10 ml of dimethyl sulfoxide (DMSO) (200 mg/ml) for methanol extract and 2g of each dry plant extract in 10 ml of sterile distilled water (200 mg/ml) for aqueous extract (16)

Test microbial isolates:

The clinical isolates of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Candida albicans* were obtained from the Medical Microbiology laboratory of the University of Ilorin

Teaching Hospital, Ilorin, Nigeria. The isolates were sub-cultured on Blood and MacConkey agar plates (bacteria) and Sabouraud dextrose agar (fungi) and incubated at 37°C for 24 hours (16).

Determination of antimicrobial activity of the plant extracts on selected pathogens:

The antimicrobial activity of the plant extracts on the selected microbial pathogens was determined by the agar well diffusion method. Each of the isolate suspension was prepared by taking a few colonies from overnight culture plate with sterilized wire loop and inoculating into normal saline. The turbidity of the bacterial suspension was adjusted to get the equivalence of 0.5 McFarland standards (~1.5×10^8 CFU/ml).

A sterile swab stick was used inoculate the microbial suspension onto the Mueller Hinton (MH) agar plate and properly spread to ensure the entire surface was covered with the inoculum. Four wells of 6 mm size each were bored in the inoculated media with sterile cork-borer. Each well was filled with 50μL of the plant extracts. DMSO was used as negative/ solvent control while positive controls (gentamicin 10μg and nitrofurantoin 100μg for bacteria and 10μg fluconazole for fungi) were tested along the test isolates by the disk diffusion method.

The extracts and DMSO were allowed to diffuse into the agar well for 30 minutes at room temperature and the plates incubated for 24 hours at 37◦C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of the extracts and DMSO. The of zone diameter of inhibition (ZDI) was observed and measured in mm. Three replicates of the experiment were carried out and the mean (and standard deviation) of the tests were calculated (15,16).

Determination of minimum inhibitory and bactericidal concentrations of the extracts:

The two-fold broth (tube) dilution assay was used to determine the minimum inhibitory concentration (MIC) of the plant extracts against the microbial pathogens. A total of 13 test tubes were prepared for the procedure. Tube 1 contained 2 ml of the undiluted plant extract from the extract stock solution (200mg/ml), while tubes 2 to 10 each contained 1 ml of nutrient broth.

A serial dilution (1 in 2) of the extract was carried out from tube 1 to tube 10 to achieve varying concentrations (200 mg/ml in tube 1 to 0.391 mg/ml in tube 10). Subsequently, 1 ml of microbial inoculum was added to each of the 10 tubes. Tubes 11, 12, and 13 served as controls (inoculum control, plant extract control, and broth control respectively). All the tubes were incubated for 24 hours, after which the tube with the lowest concentration of the extract showing no turbidity was recorded as the MIC.

The minimum bactericidal concentration (MBC) was determined by sub-culturing MIC tubes that showed no visible growth onto plain nutrient agar plate to determine the concentration that resulted in complete (99.9%) microbial kill (16).

Antimicrobial susceptibility test of synthetic antibiotics against bacteria pathogens:

The susceptibility of the same clinical microbial isolates to selected synthetic antibiotics was determined by the disk diffusion method of Bauer and Kirby (16). Six broad-spectrum antibiotics commonly prescribed by physicians in Nigeria for UTI; amoxicillin-clavulanic acid (augmentin AUG 30μg), imipenem (IMI 10µg), nitrofurantoin (NIT 100µg), ceftazidime (CAZ 30µg), cefoxitin (FOX 30µg), and gentamicin (GN 30µg) were selected for testing against bacteria, and fluconazole (FCN 10 µg) against *C. albicans*.

A sterile swab stick was used to inoculate standardized suspension of the microbial isolate onto the MH agar plate and properly spread to ensure the entire surface was covered. The antibiotic discs were then placed on the surface of the bacterial lawn and the plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured and compared with the CLSI standard chart to determine sensitivity or resistance of the microorganisms to the antibiotics (16).

Antimicrobial effects of combined synthetic antibiotics and plant extracts:

The antimicrobial effects of combination of both synthetic antibiotics and plant extracts on bacterial and fungi isolates were determined by the disc diffusion test. Standardized inoculum of overnight culture of each isolate was prepared and inoculated separately on MH agar plates using sterile swab.

Approximately 50µL of each medicinal plant extract was incorporated into each of the selected synthetic antimicrobial discs; amoxicillin-clavulanic acid (augmentin AUG 30μg), imipenem (IMI 10µg), nitrofurantoin (NIT 100 µg), ceftazidime (CAZ 30µg), cefoxitin (FOX 30µg), and gentamicin (GN 30µg) against bacteria, and fluconazole (FCN 10 µg) against *C. albicans*.

The plates were incubated at 37°C for 24 hours and the inhibitory zone diameters were measured and recorded for the combined extracts and synthetic antibiotics, and compared with the inhibitory zone diameters recorded for the individual (uncombined) synthetic antibiotic and plant extract, to determine if there are synergistic, antagonistic or indifference activities.

Antimicrobial effects of combined extracts of the two plants on microbial pathogens:

Antimicrobial activity of the combined extracts of *E. hirta* and *S. alata* on the microbial isolates was determined by agar well diffusion method. A sterile swab stick was used to inoculate standardized suspension of the overnight culture of each organism onto MH agar plate and properly spread to ensure the entire surface of the agar was covered with the inoculum. Four wells of 8 mm each were bored into the inoculated media with sterile cork-borer. Each well was labeled and filled with 100 µL of the mixture of extracts of the two plants. DMSO was used as negative/solvent control while positive control (gentamicin 10μg and nitrofurantoin 100μg for bacteria and 10μg fluconazole for fungi) were tested on the isolates by the disk diffusion method.

The extracts and DMSO were allowed to diffuse into the agar for 30 minutes at room temperature and incubated for 24 hours at 37◦C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of plant extracts/DMSO. The zone diameter of inhibition (ZDI) was observed, and measured in mm. Three replicates of the experiment were carried out and the mean (and standard deviation) were calculated (15,16).

Statistical analysis of data:

Data were analyzed using SPSS version 25.0. The Student's *t*-test was used to compare the mean zone diameter of inhibition (mean ZDI) of triplicate tests for each of the extract on the isolates, and $p<0.05$ was considered statistically significant.

Results:

The extraction yields of the plants showed that the highest yield was obtained from methanol extract of *S. alata* of 7.9% while the least yield was from aqueous extract of *E. hirta* of 5.2% (Table 1). Table 2 highlights the antimicrobial activities of the aqueous and methanol extracts of *E. hirta* and *S. alata* plants.

For *E. hirta*, methanol extracts exhibited significantly higher inhibitory activity than aqueous extracts ($p<0.05$), particularly against *P. aeruginosa* (mean ZDI: 20.3±0.577mm and 7.7±0.577mm respectively) and *C. albicans* (mean ZDI: 20.7±1.155mm and 6.0± 0.577mm respectively). Similarly, the methanol extract of *S. alata* outperformed the aqueous extract, producing significantly larger ($p<0.05$) inhibition zones against *S. aureus* (mean ZDI: 21.3±1.528mm and 6.0±1.155mm respectively) and *E. coli* (mean ZDI: 15.3 ± 0.577 mm and 7.0±1.000 mm respectively). However, both extracts exhibited comparable and limited activity against *K. pneumonia*e (mean ZDI: 7.0±1.000mm and 6.0±0.577mm respectively).

Table 3 shows the antimicrobial susceptibility of the conventional antibiotics against selected bacterial and fungi pathogens. The antibiotic with the highest inhibitory activity was imipenem on *E. coli* (ZDI: 35mm), followed by ceftazidime on *K. pneumoniae* (ZDI: 30mm). However, all the bacterial uropathogens showed low susceptibility (high resistance) to nitrofuratoin with ZDI ranges of 6-10 mm. *Candida albicans* on the other hand was susceptible to fluconazole with ZDI of 26mm.

Table 1: Percentage extract yields of *Euphorbia hirta* and *Senna alata* medicinal plants

Plants	Solvents	Weight of plants/g	Weight of extracts/g	% yield
Euphorbia hirta	Aqueous	100	5.2	5.2
	Methanol	100	6.4	6.4
Senna alata	Aqueous	100	6.0	6.0
	Methanol	100	7.9	7.9

Table 2: *In vitro* antimicrobial activity of aqueous and methanolic extract of *Euphorbia hirta and Senna alata* plants on selected bacteria and fungi pathogens

Organisms	Plants	Zone of inhibition (mm)		*t*-value	*p* value
		Aqueous (Mean ± SD)	Methanol (Mean ± SD)		
Staphylococcus aureus	*Euphorbia hirta*	11.0± 1.000	10.7 ± 0.577	1.000	0.423
	Senna alata	6.0 ± 1.155	21.3 ± 1.528	-10.094	0.010**
Escherichia coli	*Euphorbia hirta*	6.0 ± 1.528	18.0 ± 1.000	-8.000	0.015**
	Senna alata	7.0 ± 1.000	15.3 ± 0.577	-12.500	0.006**
Klebsiella pneumoniae	*Euphorbia hirta*	6.0 ± 1.155	15.0± 0.577	-15.588	0.004**
	Senna alata	6.0 ± 0.577	7.0 ± 1.000	-0.500	0.667
Pseudomonas aeruginosa	*Euphorbia hirta*	7.7 ± 0.577	20.3 ± 0.577	-19.000	0.003**
	Senna alata	7.0 ± 1.000	10.3 ± 0.577	-5.000	0.038**
Candida albicans	*Euphorbia hirta*	6.0 ± 0.577	20.7± 1.155	-16.252	0.004**
	Senna alata	6.0 ± 1.155	10.5± 1.000	-13.000	0.006**

** statistically significant at $p < 0.05$; mm=millimeter

Table 3: Antimicrobial susceptibility of some selected antimicrobials on selected urinary bacteria and fungi pathogens

Antimicrobials	Zone diameter of inhibition (mm) of microbial pathogens				
	S. aureus	***K. pneumoniae***	***E. coli***	***P. aeruginosa***	***Candida albicans***
GN (10µg)	15	26	7	26	-
IMI (10µg)	25	27	35	30	-
AUG (30µg)	16	6	9	28	-
CAZ (30µg)	13	30	8	10	-
FOX (30µg)	6	6	21	8	-
NIT (100µg)	6	6	10	6	-
FCN (10µg)	-	-	-	-	26

GN = Gentamicin, IMI = Imipenem, AUG= Amoxicillin-clavulanic acid, CAZ = Ceftazidime, FOX = Cefoxitin, NIT =Nitrofurantoin, FCN = Fluconazole

Tables 4 and 5 respectively showed the synergistic effects of *S. alata* and *E. hirta* plant extracts with some selected synthetic antibiotics. Imipenem showed the strongest synergistic activity with methanol and aqueous extracts of *S. alata* against *S. aureus* with mean ZDI of 48.3±0.577 mm and 45.6±0.577 mm respectively, followed by *P. aeruginosa* with respective mean ZDI of 47.6±0.577 mm and 39.0±1.000 mm, and the least, *K. pneumoniae* with respective mean ZDI of 19.3± 0.577 mm and 31.0±1.000 mm. The ZDI values of the extracts of *S. alata* combined with synthetic antibiotics are higher than the ZDI of the synthetic antibiotics without the extracts for *S. aureus* (ZDI: 25mm) and *P. aeruginosa* (ZDI: 30mm) in Table 3.

Ceftazidime exhibited the weakest synergistic activity with methanol and aqueous extracts of *S. alata* plant against *K. pneumoniae* (with respective mean ZDI of 11.0±1.000 mm and 31.6±0.577 mm), followed by *P. aeruginosa* (with respective mean ZDI of 24.6±0.577 mm and 10.3±0.577 mm), and the least, *S. aureus* (with respective mean ZDI of 11.6 ± 0.577 mm and 9.3±1.155 mm) (Table 4). Fluconazole also exhibited weak or no synergistic activity with methanol and aqueous extracts of *S. alata* against *C. albicans* with respective mean ZDI of 21.3±1.155 mm and 20.3±0.577 mm, which is less than the ZDI of 26 mm produced by fluconazole without the extracts (Table 3), indicating possible antagonism.

Similar to *S. alata*, imipenem showed the strongest synergistic activity with methanol and aqueous extracts of *E. hirta* plant against *P. aeruginosa* (with respective mean ZDI of 43.3±1.15 mm and 42.7±1.15 mm), followed by *S. aureus* (with respective mean ZDI of 41.3±1.15 mm and 38.3±1.15 mm), *K. pneumoniae* (with respective mean ZDI of 40.3±0.58 mm and 40.0±1.00 mm), and the least *E. coli* (with respective mean ZDI of 20.0±1.00 mm and 20.7±1.53 mm) (Table 5). The ZDI values of the extracts of *E. hirta* combined with synthetic antibiotics are higher than the ZDI of the synthetic antibiotics without the extracts for *S. aureus* (ZDI: 25mm) and *P. aeruginosa* (ZDI: 30mm) in Table 3.

However, fluconazole exhibited weak synergistic or indifference activity with methanol and aqueous extracts of *E. hirta* against *C. albicans* with respective mean ZDI of 30.3± 0.58 mm and 26.7±1.15 mm, which was just slightly higher than the ZDI of 26 mm produced by fluconazole without the extracts (Table 3) for the methanol extract, but equivalent to that produced by aqueous extract, indicating possible indifference activity.

Table 4: Synergistic effect of *Senna alata* plant with selected synthetic antimicrobials on urinary bacteria and fungi pathogens

Antimicrobial	Organism	Zone of inhibition (mm)		*t*-value	*p*-value
		Aqueous (Mean ± SD)	Methanol (Mean ± SD)		
GN	*S. aureus*	13.5 ± 0.61	22.3 ± 1.53	-9.23	0.0009**
	K. pneumoniae	15.0 ± 1.17	25.0± 1.00	-12.01	0.0005**
	E. coli	16.2 ± 1.18	21.9 ± 1.23	-4.26	0.0112
	P. aeruginosa	25.0 ± 1.00	31.1 ± 0.61	-10.21	0.0003**
IMI	*S. aureus*	45.6 ± 0.577	48.3 ± 0.577	-4.000	0.057
	K. pneumoniae	31.0 ± 1.000	19.3 ± 0.577	35.000	0.001**
	E. coli	32.7 ± 0.577	31.3 ± 1.528	1.512	0.270
	P. aeruginosa	39.0 ± 1.000	47.6 ± 0.577	-13.000	0.006**
AUG	*S. aureus*	19.3 ± 1.155	20.7 ± 0.577	-4.000	0.057
	K. pneumoniae	40.3 ± 0.577	38.0 ± 1.000	7.000	0.020**
	E. coli	31.0 ± 1.000	33.6 ± 0.577	-3.024	0.094
	P. aeruginosa	21.3 ± 1.155	27.3 ± 0.577	-10.392	0.009**
CAZ	*S. aureus*	9.3 ± 1.155	11.6 ± 0.577	-7.000	0.020**
	K. pneumoniae	31.6 ± 0.577	11.0 ± 1.000	62.000	0.000**
	E. coli	15.3 ± 0.577	14.0 ± 0.000	4.000	0.057
	P. aeruginosa	10.3 ± 0.577	24.6 ± 0.577	-43.000	0.001**
FOX	*S. aureus*	23.6 ± 0.577	21.0 ± 1.000	4.000	0.057
	K. pneumoniae	30.3 ± 17.616	27.3 ± 1.155	0.285	0.803
	E. coli	18.3 ± 0.577	14.0 ± 1.000	13.000	0.006**
	P. aeruginosa	21.3 ± 1.528	25.0 ± 1.000	-3.051	0.093
NIT	*S. aureus*	11.3 ± 0.59	12.6± 0.63	0.00	1.0000
	K. pneumoniae	7.0 ± 1.15	7.0± 1.15	0.47	0.6769
	E. coli	14.0 ± 0.58	14.3 ± 0.58	-2.17	0.1012
	P. aeruginosa	12.4 ± 0.45	16.1 ± 0.72	-7.83	0.0013**
FCN	*Candida albicans*	20.3 ± 0.577	21.3 ± 1.155	-1.000	0.423

GN = Gentamicin, IMI = Imipenem, AUG = Amoxicillin-clavulanic acid, CAZ = Ceftazidime, FOX = Cefoxitin, NIT =Nitrofurantoin, FCN = Fluconazole; ** mean significance at *p*-value < 0.05; SD: Standard deviation

Table 5: Synergistic effects of *Euphorbia hirta* with selected synthetic antimicrobials on urinary bacterial and fungi pathogens

Antimicrobials	Organism	Zone of inhibition (mm)		*t*-value	*p*-value
		Aqueous (Mean ± SD)	Methanol (Mean ± SD)		
GN	*S. aureus*	11.7 ± 0.58	20.3 ± 1.53	-9.19	0.0008**
	K. pneumoniae	12.0 ± 1.00	21.0 ± 1.00	-11.02	0.0004**
	E. coli	16.7 ± 1.15	20.7 ± 1.15	-4.24	0.0132**
	P. aeruginosa	21.0 ± 1.00	27.7 ± 0.58	-10.00	0.0006**
IMI	*S. aureus*	38.3 ± 1.15	41.3 ± 1.15	-3.18	0.0335**
	K. pneumoniae	40.0 ± 1.00	40.3 ± 0.58	-0.50	0.6433
	E. coli	20.7 ± 1.53	20.0 ± 1.00	0.63	0.5614
	P. aeruginosa	42.7 ± 1.15	43.3 ± 1.15	-0.71	0.5185
AUG	*S. aureus*	21.0 ± 1.00	19.7 ± 0.58	2.00	0.1161
	K. pneumoniae	40.3 ± 0.58	18.0 ± 1.00	33.50	0.0000**
	E. coli	25.3 ± 0.58	25.7 ± 0.58	-0.71	0.5185
	P. aeruginosa	20.7 ± 0.58	25.3 ± 1.15	-6.26	0.0033**
CAZ	*S. aureus*	10.3 ± 0.58	9.0 ± 1.00	2.00	0.1161
	K. pneumoniae	18.0 ± 1.00	23.7 ± 0.58	-8.50	0.0011**
	E. coli	6.7 ± 1.15	6.3 ± 0.58	0.45	0.6779
	P. aeruginosa	10.3 ± 0.58	34.0 ± 1.00	-35.50	0.0000**
FOX	*S. aureus*	21.0 ± 1.00	23.0 ± 1.00	-2.45	0.0705
	K. pneumoniae	27.3 ± 1.15	22.3 ± 1.53	4.52	0.0106**
	E. coli	11.3 ± 0.58	5.7 ± 0.58	12.02	0.0003**
	P. aeruginosa	31.0 ± 1.00	30.3 ± 0.58	1.00	0.3739
NIT	*S. aureus*	10.7 ± 0.58	10.6 ± 0.58	0.00	1.0000
	K. pneumoniae	6.6 ± 1.15	6.3 ± 0.58	0.45	0.6779
	E. coli	13.3 ± 0.58	14.3 ± 0.58	-2.12	0.1012
	P. aeruginosa	10.6 ± 0.58	14.3 ± 0.58	-7.78	0.0015**
FCN	*Candida albicans*	26.7 ± 1.15	30.3 ± 0.58	-4.92	0.0079**

GN = Gentamicin, IMI = Imipenem, AUG = Amoxicillin-clavulanic acid, CAZ = Ceftazidime, FOX = Cefoxitin, NIT =Nitrofurantoin, FCN = Fluconazole; ** mean significance at *p*-value < 0.05; SD: Standard deviation

The antimicrobial activity of the combined extracts of *E. hirta* and *S. alata* on the pathogens in Table 6 showed that methanol extracts of the combined plants exhibited higher inhibitory activity than aqueous extracts against most of the tested pathogens, producing significantly higher ZDI against *E. coli* (mean ZDI: 24.0±1.000 mm), *K. pneumoniae* (mean ZDI: 20.3±0.577 mm), and *C. albicans* (mean ZDI: 21.0±1.155 mm), compared to the lower ZDI of the aqueous extracts of 6.3± 0.577 mm, 6.7±1.155 mm and 6.3±0.577 mm for the respective pathogens. There was no difference in the inhibitory activity of the combined methanol and aqueous extracts of the two plants against *S. aureus* (mean ZDI: 19.3±0.577 and 16.0±0.577 mm respectively) and against *P. aeruginosa* (mean ZDI: 20.3 ±0.577 and 20.7±0.577 mm, respectively).

Table 7 presents the MIC values of *S. alata* extracts against the selected pathogens, with methanol extracts producing lower MIC values (range 6.25–50 mg/ml) than aqueous extracts (25-100 mg/ml). The lowest MIC values with methanol extracts of *S. alata* were produced against *C. albicans* (6.25 mg/ml) and *S. aureus* (12.5mg/ml), followed by MIC values of 50 mg/ml against *P. aeruginosa*, *E. coli* and *K. pneumoniae*. However, high MIC of 100 mg/ml was produced with aqueous extracts of *S. alata* against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*.

The methanol extracts of *E. hirta* produced lower MIC values (range 6.25–50 mg/ml) than aqueous extracts (range 50-100 mg/ml). The lowest MIC values with methanol extracts of *E. hirta* were produced against *P. aeruginosa* (6.25 mg/ml) and *C. albicans* (12.5 mg/ml), followed by *K. pneumoniae* (25 mg/ml), *E. coli* (25 mg/ml) and *S. aureus* (50 mg/ml). Comparatively, aqueous extracts of *E. hirta* produced high MIC values against *P. aeruginosa* (25 mg/ml), *E. coli* (50 mg/ml), *S. aureus* (50 mg/ml) and *K. pneumoniae* (100 mg/ml).

Table 8 presents the MBC values of *E. hirta* against some selected pathogens. The MBC values for methanol extracts range from 25 mg/ml against *P. aeruginosa* to 100 mg/ml against *S. aureus* while MBC values for aqueous extract range from 100 mg/ml against *C. albicans* to 200 mg/ml against *S. aureus.* For *S. alata*, the MBC values of the methanol extracts range from 25 mg/ml against *C. albicans* to 100 mg/ml against *K. pneumoniae* while the MBC values of the aqueous extracts range from 100 mg/ml against *C. albicans* to 200 mg/ml against *K. pneumoniae.*

Table 6: *In vitro* antimicrobial activity of aqueous and methanolic extracts of combined *Euphorbia hirta* and *Senna alata* plants on selected urinary bacteria and fungi pathogens

Organism	**Zone of inhibition (mm)**		***t*-value**	***p*-value**
	Aqueous (Mean ± SD)	**Methanol (Mean ± SD)**		
Staphylococcus aureus	16.0 ± 0.577	19.3 ± 0.577	-8.000	0.015**
Escherichia coli	6.3 ± 0.577	24.0 ± 1.000	-26.500	0.001**
Klebsiella pneumoniae	6.7 ± 1.155	20.3 ± 0.577	-41.000	0.001**
Pseudomonas aeruginosa	20.7 ± 0.577	20.3 ± 0.577	1.000	0.423
Candida albicans	6.3 ± 0.577	21.0± 1.155	-43.000	0.001**

mean significance at p-value < 0.05; mm = millimeter

Table 7: Minimum Inhibitory Concentration (MIC) of *Euphorbia hirta* and *Senna alata* extract against selected urinary bacteria and fungi pathogens

Organism	***Euphorbia hirta***		***Senna alata***	
	Aqueous Extract	**Methanolic Extract**	**Aqueous Extract**	**Methanolic Extract**
Staphylococcus aureus	50 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml
Klebsiella pneumoniae	100 mg/ml	25 mg/ml	100 mg/ml	50 mg/ml
Escherichia coli	50 mg/ml	25 mg/ml	50 mg/ml	50 mg/ml
Pseudomonas aeruginosa	25 mg/ml	6.25 mg/ml	25 mg/ml	25 mg/ml
Candida albicans	50 mg/ml	12.5 mg/ml	50 mg/ml	6.25 mg/ml

Table 8: Minimum Bactericidal Concentration (MBC) of *Euphorbia hirta* and *Senna alata* extract against selected urinary bacteria and fungi pathogens

Organism	***Euphorbia hirta***		***Senna alata***	
	Aqueous Extract	**Methanolic Extract**	**Aqueous Extract**	**Methanolic Extract**
Staphylococcus aureus	200 mg/ml	100 mg/ml	200 mg/ml	100 mg/ml
Klebsiella pneumoniae	200 mg/ml	100 mg/ml	200 mg/ml	100 mg/ml
Escherichia coli	200 mg/ml	100 mg/ml	100 mg/ml	100 mg/ml
Pseudomonas aeruginosa	100 mg/ml	25 mg/ml	200 mg/ml	100 mg/ml
Candida albicans	100 mg/ml	50 mg/ml	100 mg/ml	25 mg/ml

Discussion:

The extraction yield of the leaves of the medicinal plants analyzed in this study showed that the highest yield was obtained from methanol extracts of *S. alata* (7.9%) with methanol extracts of *E. hirta* producing a lower yield of 6.4%. The yields with aqueous extracts were correspondingly lower than the methanol extracts for the two plants. This variation is primarily attributable to the nature of the solvent used, which significantly affects the solubility and extractability of phytochemicals in the plants. Polar solvents such as water are more selective for hydrophilic compounds, whereas solvents such as methanol, with intermediate polarity, can extract a broader range of both polar and non-polar constituents, resulting in higher yields. These findings align with previous studies (17,18) that reported similar solvent-dependent variations in extractive values of medicinal plants. Similarly, Tourabi et al., (19) reported that the extraction yield of methanol on medicinal plants generally exceeded that of aqueous extracts due to the superior solvent penetration ability of methanol.

Methanol extracts of *E. hirta* in our study demonstrated significantly higher antimicrobial activity than aqueous extracts, particularly against *K. pneumoniae*, *E. coli*, *C. albicans* and *P. aeruginosa.* Similarly, methanol extracts of *S. alata* exhibited higher inhibitory activity against *S. aureus* and *E. coli*. These findings are consistent with those of previous studies (20,21) which reported that methanol extracts of medicinal plants exhibited higher antimicrobial activity than aqueous extracts. The higher antimicrobial activity of methanol extracts could be attributed to greater solubility of the active phytochemicals in methanol, leading to enhanced activity (22).

The study on the combined antimicrobial effects of *E. hirta* and *S. alata* shoed both synergistic and antagonistic interactions against various pathogens. The methanolic extract of *S. alata* exhibited antagonistic effects when combined with *E. hirta* against *S. aureus*, as the inhibition zone decreased from 21 mm (for *S. alata* alone) to 19 mm in the combination. Conversely, synergistic effects were observed against *E. coli* and *K. pneumoniae*, with inhibition zones increasing from 18 mm to 24 mm and 15 mm to 20 mm, respectively. These findings contrast with previous studies (16,23), which reported strong synergistic interactions across all tested combinations of medicinal plant extracts and no antagonistic effects. The observed variation may be attributed to differences in the specific plant species used, extraction methods, phytochemical compositions, or microbial strains used in the studies. Adal et al., (24) similarly reported that *S. alata* extracts were effective against both Gram-positive and Gram-negative bacteria, particularly when using organic solvents. Their findings aligns with the results of the current study regarding antimicrobial activity of *S. alata* extracts against *S. aureus* and *P. aeruginosa*.

The *in vitro* antimicrobial susceptibility of the selected pathogens to the antimicrobial agents commonly used for the treatment UTIs showed significant insights into the effectiveness of the various antibiotics against these uropathogens in our study. Imipenem was identified as the most potent antibiotic against all tested pathogens, which is consistent with findings from other studies that emphasize its effectiveness against multidrug-resistant bacteria (25,26). However, the growing concern of resistance to carbapenems, particularly in hospital settings, has been documented in other studies, indicating that while imipenem remains effective, its use should be monitored closely due to potential resistance development (27).

The patterns of the pathogen susceptibility observed align with previous research, highlighting the challenges in managing UTIs due to varying resistance patterns (28). Imipenem and gentamicin demonstrated minimal activity against *S. aureus*, with zone diameter of inhibition (ZDI) of 25 mm and 15 mm respectively, consistent with findings of Rahim et al., (29) who reported significant resistance of *S. aureus* to these antibiotics. *K. pneumoniae* were largely susceptible to imipenem (ZDI: 27mm) and gentamicin (ZDI: 26mm), reinfor-

cing their effectiveness as treatment options (30,31). Similarly, ceftazidime (ZDI: 30 mm) showed strong activity against *K. pneumoniae* as observed in other studies (32). *E. coli* demonstrated the highest susceptibility to imipenem (ZDI: 35 mm), further confirming its potency against this common uropathogen. *P. aeruginosa* exhibited susceptibility to gentamicin (ZDI: 26 mm) and imipenem (ZDI: 26 mm), supporting the literature that identifies these antibiotics as first-line treatments (36). Fluconazole showed high inhibitory activity against *C. albicans* (ZDI: 26 mm), although susceptibility to fluconazole has been reported to vary by strain of *Candida* and location (37). Overall, imipenem was the most potent antibiotic against all tested bacterial pathogens, consistent with other studies, highlighting its role against multidrug-resistant strains (38). However, the increasing concern of carbapenem resistance, particularly in the hospital settings, underscores the need for careful monitoring of its use (39).

Synergistic testing of the plant extracts combined with conventional antibiotics evaluated in this study revealed notable synergistic interactions, particularly with *S. alata* extracts. Among the antibiotics tested, imipenem exhibited the highest synergistic activity when combined with both the methanolic and aqueous extracts of *S. alata*, especially against *S. aureus*, where the inhibition zones markedly increased from 25mm (for imipenem disc alone) to 48 mm and 46 mm, respectively. Amoxicillin-clavulanic acid also displayed substantial synergistic effects in combination with *S. alata* extracts with inhibition zones increasing from 6-28 mm (for amoxicillin-clavulanic acid alone) to 19-40 mm when combined with the plant extracts. Gentamicin showed mild synergism ranges from 13-31 mm compared to 15-26 mm. Similarly, cefoxitin demonstrated synergism with inhibition zones ranging from 6-20 mm alone and increasing to 19-30 mm when combined with *S. alata* extracts.

In contrast, ceftazidime demonstrated weaker synergistic or indifference effects across all tested bacterial pathogens, with inhibition zones ranging from 8-30 mm for ceftazidime disc alone, and 9-31 mm when combined with *S. alata* extract. Nitrofurantoin also exhibited weak synergistic effects, with inhibition zones increasing slightly from 6-10 mm to 7-16 mm across all pathogens. The anti-fungi drug, fluconazole, when combined with *S. alata* extracts, exhibited antagonistic interaction against *C. albicans*, as the inhibition zone decreased from 26 mm to 21 mm.

Synergistic activity was also observed when imipenem was combined with both methanolic and aqueous extracts of *E. hirta*, particularly against *P. aeruginosa* (inhibition zone of 43 mm for both extracts) and *S. aureus* (41 mm and 38 mm for methanol and aqueous extracts respectively), but no synergistic effect was observed against *E. coli*, with inhibition zones of 20 mm and 21 mm, respectively. Interestingly, amoxicillin-clavulanic acid in combination with *E. hirta* also showed consistent synergism across all tested pathogens, with inhibition zones ranging from 18-40 mm. Cefoxitin showed inhibition zones of 6-31 mm, nitrofurantoin 6-14 mm, gentamicin 11-28 mm, and ceftazidime 6-34 mm when combined with *E. hirta* extracts. Contrastingly to *S. alata* extracts, fluconazole combined with *E. hirta* extracts showed enhanced synergistic activity against *C. albicans*, with inhibition zones increasing from 26 mm to 30 mm. These findings are consistent with those reported by Alam et al., (40) and Acharjee et al., (41) who in separate studies reported enhanced antimicrobial activities when plant extracts were combined with standard antibiotics. The observed synergistic interactions highlight the therapeutic potential of integrating medicinal plant extracts with conventional antibiotics as a strategy to enhance antimicrobial efficacy and mitigate the global issue of antibiotic resistance.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results further reinforce the potency of methanol extracts over aqueous extracts. The methanol extracts of *E. hirta* methanol extract produced lower MIC values, ranging from 6.25 mg/ml against *P. aeruginosa* to 50mg/ml against *S. aureus*, whereas aqueous extracts ranged from 25mg/ml to 100mg/ml. These results agree with the study by Mehmood et al., (42), who reported similar trends in lower MIC values for methanol extracts of medicinal plants. Similarly, methanol extract of *S. alata* produced MIC values as low as 6.25 mg/ml against *C. albicans* but as high as 50mg/ml for *K. pneumoniae* and *E. coli*, contrasting with aqueous extracts which produced very high MIC values of up to 100 mg/ml.

These findings agree with the study of other researchers (42,43), who reported that methanol extracts of medicinal plants tend to produce lower MIC and MBC values compared to aqueous extracts. The MBC test showed that methanol extracts of both *E. hirta* and *S. alata* exhibited stronger bactericidal effects compared to aqueous extracts, with MBC of the methanol extract of *E. hirta* ranging from 25mg/ml against *P. aeruginosa* to 100mg/ml against *S. aureus*, while aqueous extracts ranged from 100mg/ml for *C. albicans* to 200 mg/ml for *S. aureus*. Similarly, methanol extract of *S. alata* produced MBC values that ranged from 25 mg/ml for *C. albicans* to 100 mg/ml for *K. pneumoniae*, whereas the aqueous extracts ranged from 100mg/ml for *C. albicans* to 200mg/ml for *K. pneumoniae*. These findings agree with previous studies, demonstrating the superiority of methanol ex-

tracts in extracting bioactive compounds responsible for antimicrobial activity. For example, Ghaffar and Perveen (44) reported that methanol extracts of medicinal plants often produce lower MBC values due to their ability to dissolve both polar and non-polar phytochemicals, unlike aqueous extracts that mainly extract polar compounds. Furthermore, Mosallaie et al., (45) reported that phenolics and alkaloids, commonly extracted by methanol, are crucial contributors to bactericidal activity, supporting our findings in this study.

Our study has some limitations. First, the study was entirely an *in vitro* evaluation, which may not fully reflect the complex interactions that occur within a living organism. The observed antimicrobial and synergistic effects may differ *in vivo* due to factors such as bioavailability, metabolism, and host immune system. Second, our study utilized crude methanol and aqueous extracts, which contain a mixture of several phytochemicals. This may mask the individual effect of specific bioactive compounds, and difficulty in identifying the potential synergistic or antagonistic interactions among the phytochemicals.

Conclusion:

Nevertheless, this study highlights the significant antimicrobial properties of both methanol and aqueous extracts of *S. alata* and *E. hirta*. The methanol extracts consistently exhibited higher extraction yields and greater antimicrobial activities compared to the aqueous extracts. Furthermore, the observed synergistic interactions with antibiotics such as imipenem underscore the therapeutic potentials of these medicinal plants, especially in the context of combating antimicrobial resistant pathogens.

Contributions of authors:

BRT carried out the research and wrote the draft of the manuscript; DZA, MZK, SHB, SHU and YF collected the samples and prepared them for extraction; OBO supervised the project and revised the manuscript; EAH co-supervised the project and revised the manuscript; AM and MTO carried out the statistical analysis. All authors read and approved the manuscript submitted for publication.

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References:

1. Alara, J. A., and Alara, O. R. An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis of infectious disorders. Drug Targets Disorders. 2024; 24 (3): 26-42. doi 10.2174/1871526523666230725103902
2. Baimakhanova, B., Sadanov, A., Trenozhnikova, L., et al. Understanding the Burden and Management of Urinary Tract Infections in Women. Diseases 2025; 13 (2): 59. doi: 10.3390/diseases13020059
3. Lin, W., Ruishi, X., Caijiao, X., et al. Potential applications and mechanisms of natural products in mucosal-related diseases. Front Immunol. 2025; 16: 1594224 doi: 10.3389/fimmu.2025.1594224
4. Aththanayaka, A. M. W. G. K. P., Weerasinghe, G. G. Y. H., Weerakkody, N. S., Samarasinghe, S. H. G. G., and Priyadharshana, U. Effectiveness of selective antibiotics use in ESBL-related UTIs. BMC Microbiol. 2024; 24 (1): 360. doi: 10.1186/s12866-024-03495-y
5. Ekpunobi, N., Ugwu, M., Agu, K., et al. Antimicrobial Resistance in Pediatric UTIs: Molecular Profiles, Clinical Burden, and Public Health Implications of ESBL-Producing Uropathogens. 2025 doi:10.20944/preprints202505.2019.v1
6. World Health Organization. No time to Wait: Securing the future from drug-resistant infections. Report to the Secretary-General of the United Nations. WHO 2019
7. Chaachouay, N., and Zidane, L. Plant-derived natural products: a source for drug discovery and development. Drugs and Drug Candidates. 2024; 3 (1): 184-207. https://doi.org/10.3390/ddc3010011
8. Nagarajan, P., Kannan, S., Raj, A., et al. Future Prospects in Medicinal Plant-Derived Drug Development: Unveiling Opportunities and Challenges. In: Biotechnology and Phytochemical Prospects Drug Discovery. Springer. 2025; 241-266. doi:10.1007/978-981-96-2790-5_9
9. Singh, P. A., Dash, S., Choudhury, A., and Bajwa, N. Factors affecting long-term availability of medicinal plants in India. J Crop Sci Biotechnol. 2024; 27 (2): 145-173. doi:10.1007/s12892-023-00219-y
10. Hilal, B., Khan, M. M., and Fariduddin, Q. Recent advancements in deciphering the therapeutic properties of plant secondary metabolites: phenolics, terpenes, and alkaloids. Plant Physiol Biochem. 2024; 108674. doi: 10.1016/j.plaphy.2024.108674
11. Sarwar, R., Maisam, M., Khan, M. W., Xue, Y., and Hassan, S. Bioactive Compounds in Herbal Remedies. In: Herbal Pharmacopeia. CRC Press. 2025: 89-121.
12. Bamidele, H. A., Umar, A., Tanko, H. O., Adegboye, Y., Obuchi, A., and Kazeem, F. A. Uses and Health Benefits of Medicinal Plants. Bijote-Bichi J Technol Education. 2024; 7 (1): 39-44.
13. Tama, R. T., Hossain, M. S., Rahaman, M. A., et al. Harnessing the Power of Natural Products Against Bacterial Urinary Tract Infections: A Perspective Review for Cultivating Solutions. Health Sci Rev. 2024: 13: 100199. https://doi.org/10.1016/j.hsr.2024.100199
14. Irawan, C., Hindarto, C. K., Enriyani, R., Sukiman, M., and Prastiwi, B. Effect of extraction effectivity of *Senna alata* L. leaves on the total phenolic, antioxidative, antimicrobial and anti-goat activity. Res J Pharm Technol. 2024; 17 (3): 997-1002. doi:10.52711/0974-360x.2024.00154
15. Hung, N. Q., Ha, L. T. M., Lien, D. T., Nga, N. T. T., and Lam, V. P. Optimal shoot mass for

propagation to increase the yield and quality of pineapple. Sustainability. 2024; 16 (13): 5729. https://doi.org/10.3390/su16135729
16. Bakir Çilesizoğlu, N., Yalçin, E., Çavuşoğlu, K., and SipahiKuloğlu, S. Qualitative and quantitative phytochemical screening of *Nerium oleander* L. extracts associated with toxicity profile. Sci Rep. 2022; 12 (1): 21421 doi 10.1038/s41598-022-26087-0
17. Nzor, J. N., Uwakwe, A. A., and Ogunka-Nnoka, C. U. Comparative analysis of *Anthocleista vogelii* leaf extracts: solvent influence on phytochemical composition, quantitative profile and *invitro* antioxidant activities. Int J Innov Biochem Microbiol Res. 2024; 12: 1-7.
18. Lai, Y. J., Chiang, Y. C., Jhan, Y. S., Song, T. Y., and Cheng, M. C. Extraction Effects on Roselle Functionalities: Antioxidant, Antiglycation, and Antibacterial Capacities. Foods. 2024; 13 (14): 2172. https://doi.org/10.3390/foods13142172
19. Tourabi, M., Faiz, K., Ezzouggari, R., et al. Optimization of extraction process and solvent polarities to enhance the recovery of phytochemical compounds, nutritional content, and bio-functional properties of *Mentha longifolia* L. extracts. Bioresources and Bioprocessing. 2025; 12 (1): 24. doi: 10.1186/s40643-025-00859-8
20. Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S., and Mustafa, A. *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chem. 2022; 16 (1): 64. doi: 10.1186/s13065-022-00858-2.
21. Alemu, M., Lulekal, E., Asfaw, Z., et al. Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. PLoS One. 2024; 19 (3): e0300060. doi: 10.1371/journal.pone.0300060
22. Mwangi, W. C., Waudo, W., Shigwenya, M. E., and Gichuki, J. Phytochemical characterization, antimicrobial and antioxidant activities of *Terminalia catappa* methanol and aqueous extracts. BMC Complem Med Ther. 2024; 24 (1): 137. doi: 10.1186/s12906-024-04449-7
23. Shahin, H. H., Baroudi, M., Dabboussi, F., et al. Synergistic Antibacterial Effects of Plant Extracts and Essential Oils Against Drug-Resistant Bacteria of Clinical Interest. Pathogens. 2025; 14 (4): 348. doi: 10.3390/pathogens14040348
24. Adal, T. K., Endalew, S. A., Beshir, A. B., and Wondimagegn, M. K. Evaluation of Antibacterial Activity of a Sesquiterpenoid Isolated from the Leaves of *Laggera tomentosa* Endemic to Ethiopia. Nat Sci. 2025; 5 (1-2): e70006. doi:10.1002/ntls.70006
25. Kumar, A., Kumar, M., Jose, A., Tomer, V., Oz, E., and Oz, F. Major phytochemicals: Recent advances in health benefits and extraction method. Molecules. 2023; 28 (2): 887. doi: 10.3390/molecules28020887
26. Vaou, N., Stavropoulou, E., Voidarou, C., et al. Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. Antibiotics. 2022 ; 11 (8): 1014. doi: 10.3390/antibiotics11081014
27. Toh, S. C., Lihan, S., Bunya, S. R., and Leong, S. S. *In vitro* antimicrobial efficacy of *Cassia alata* (Linn.) leaves, stem, and root extracts against cellulitis causative agent *Staphylococcus aureus* BMC Complement Med Ther. 2023; 23 (1): 85. doi: 10.1186/s12906-023-03914-z
28. Dbeibia, A., Taheur, F. B., Altammar, K. A., et al. Control of *Staphylococcus aureus* methicillin resistant isolated from auricular infections using aqueous and methanolic extracts of Ephedra alata. Saudi J Biol Sci. 2022; 29 (2): 1021-1028. doi: 10.1016/j.sjbs.2021.09.071
29. Rahim, N. A., Ferdosh, S., Zainuddin, N. A. N. B., and Sarker, M. Z. I. Extraction Methodologies, Phytochemical Constituents, and Biological Activities of *Senna alata* Linn: A Review. Nat Prod J. 2023; 13 (2): 2-18. doi:10.2174/2210315512666220427114719
30. Nguyen, M., and Joshi, S. G. Carbapenem resistance in *Acinetobacter baumannii*, and their importance in hospital-acquired infections: a scientific review. J Appl Microbiol. 2021; 131 (6): 2715-2738. doi: 10.1111/jam.15130
31. Que, A. T., Tran, A. D., Trang, T. H. N., Tran, T. N. L., Bui, N. N., and Lai, C. H. Epidemiology and antimicrobial resistance patterns of urinary tract infection: insights and strategies from a 5-year serial cross-sectional study in Vietnam. Therap Adv Infect Dis. 2025; 12 20499361251315346. doi: 10.1177/20499361251315346
32. Abebe, R. Antimicrobial susceptibility and resistance patterns of *Staphylococcus aureus* isolated from mastitic cows in Hawassa city and its suburbs, southern Ethiopia. J Res Vet Sci. 2025; 5 (1): 41-41. doi: 10.5455/JRVS.20241216112919
33. Ramadan, R. A., Bedawy, A. M., Negm, E. M., et al. Carbapenem-resistant Klebsiella pneumoniae among patients with ventilator-associated pneumonia: Evaluation of antibiotic combinations and susceptibility to new antibiotics. Infect Drug Res. 2022; 3537-3548. doi: 10.2147/IDR.S371248
34. Algfri, S. K., Naser, G. A., Rageh, R. S., Shuaib, A., and Nasser, A. H. Anatomical, Physicochemical, Phytochemical, Antioxidant and Antimicrobial Investigations of *Tagetes minuta* L. aerial parts. Electronic Journal of University of Aden for Basic and Applied Sciences. 2024; 5 (4): 507-520.
35. Taha, R., Kader, O., Shawky, S., and Rezk, S. Ceftazidime-Avibactam plus aztreonam synergistic combination tested against carbapenem-resistant Enterobacterales characterized phenotypically and genotypically: a glimmer of hope. Ann Clin Microbiol Antimicrob. 2023; 22 (1): 21. doi: 10.1186/s12941-023-00573-3
36. Kincses, A., Rácz, B., Baaity, Z., et al. The relationship between antibiotic susceptibility and pH in the case of uropathogenic bacteria. Antibiotics. 2021; 10 (12): 1431. doi: 10.3390/antibiotics10121431
37. Subedi, A., Upreti, M. K., Rana, J. C., Sapkota, R. P., and Shrestha, U. T. Vulvovaginal candidiasis, an increasing burden to women in the tropical regions attending Bharatpur Hospital, Chitwan. J Med Mycol. 2024; 34 (4): 101509. doi: 10.1016/j.mycmed.2024.101509
38. Terreni, M., Taccani, M., and Pregnolato, M. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules. 2021; 26 (9): 2671. doi: 10.3390/molecules26092671
39. Laddad, M., Kanase, N. V., and Mohite, R. V. The Challenge of Carbapenem Resistance: Strategies for Prevention and Control. Obstetrics and Gynaecology Forum. 2024; 34 (3s): 385-392
40. Alam, M., Bano, N., Ahmad, T., et al. Synergistic role of plant extracts and essential oils against multidrug resistance and gram-negative bacterial strains producing extended-spectrum β-lactamases. Antibiotics. 2022; 11(7): 855. doi: 10.3390/antibiotics11070855
41. Acharjee, M., Hasan, F., Islam, T., Nur, I. T., Begum, N., and Mazumder, C. *In vitro* antibacterial activity of commercially available probiotics on foodborne pathogens along with their synergistic effects with synthetic drugs. Am J Infect Dis. 2022; 21: 100-187. doi: 10.1016/j.metop.2022.100187
42. Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S., and Mustafa, A. *In vitro* total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chem. 2022; 16 (1): 64. doi: 10.1186/s13065-022-00858-2
43. López González, R., Gutiérrez, M. H., García, R. L., et al. ZnO nanomaterials with enhanced antimicrobial activity obtained by *Eichhornia*

crassipes aqueous extract-mediated synthesis. J Chem Technol Biotechnol. 2024; 99 (12): 2535-2544. doi:10.1002/jctb.7601

44. Ghaffar, N., and Perveen, A. Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study. NZ J Botany. 2024: 63 (11): 1-11. doi:10.1080/0028825x.2024.2392705

45. Mosallaie, F., Pirnia, M., Dehghan, Z., et al. Unveiling the chemical composition, antioxidant and antibacterial properties, and mechanistic insights of *Convolvulus arvensis* extract through molecular docking simulations. Appl Food Res. 2024; 4 (2): 100580. https://doi.org/10.1016/j.afres.2025.100707