

Enhanced Combined Antibacterial Action of *Coriandrum sativum*, *Murraya koenigii*, and *Piper nigrum* Against Uropathogenic Bacterial Strains

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Abstract

Urinary tract infections (UTIs) are among the most common, serious health issues mainly caused by bacteria, which are increasingly developing resistance. Therefore, researchers are focused to find novel plant derived antimicrobials amid raised treatment concerns among healthcare facilities. The purpose of this study is to evaluate the antibacterial properties of plants *Coriandrum sativum*, *Murraya koenigii* and *Piper nigrum* individually and in combinations (1:1 and 1:1:1) along with their total phenolic (TPC) and total flavonoid content (TFC). It is an experimental study where n-hexane, ethyl acetate, methanolic and distilled water (dH₂O) extract's minimum inhibitory concentrations (MICs), and minimum bactericidal concentrations (MBCs) were determined against *Acinetobacter baumannii* ATCC 45269, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 9528 strains. Microsoft excel pro365, was utilized to calculate mean and standard error mean (SEM) with P<0.05 considered significant. Largest inhibition zones (7-10.33 mm IZs) were observed against *A. baumannii*, for individual extracts, whereas MICs for combinations were (12.5µg/mL-100 µg/mL) against test strains, with lowest MIC (12.5 µg/mL) for water pepper-curry extract (WPCu) against *E. coli*. Significant TPC and TFC values were observed for methanolic (29.36 and 32.32 mg GAE/g and 15.048 and 17.563 mg QE/g) and water (31.16 and 28.72 mg GAE/g, and 18.922 and 14.736 mg QE/g) extracts of *P. nigrum* and *M. koenigii* respective. The results suggested that these plants have potential attributes against uro-bacteria in combinations and hence could be used as alternative for formulations for UTI.

Keywords: Antibiotic resistance, Combination activities, Plant antimicrobials, UTIs, Uro-pathogenic bacteria

1. Introduction

Urinary tract infections (UTIs) represent the second most common type of infection worldwide, which can occur among 10% people in a lifetime (Farajnia, 2009), with prominent mortality and morbidity at 1-2 years of age (Singh and Madhup, 2013). Gram-negative and Gram-positive bacterial species which include *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis* and significantly *Escherichia coli*, are responsible for 95% UTIs (Farajnia, 2009). Rapid accurate diagnosis followed by empirical treatment is a key for reduction in disease manifestation which is generally decided by causative pathogen's resistance to antibiotics (Mirsoleymani *et al.*, 2014). However, concerns are arising in Pakistan and globally due to emerging antibiotic resistance among these species (Ahmad *et al.*, 2020) particularly due to irrationally selected and administered antibiotics for treatment (Kowalska-Krochmal and Dudek-Wicher, 2021). *Enterobacteriaceae* family is of most concern regarding antibiotic resistance, especially cephalosporin resistance (Chen *et al.*, 2013; Paterson, 2006). Uropathogens are also becoming multidrug resistant (MDR) which is a stimulant for developing substitutive therapeutics and control measures (Gupta and Bhadelia, 2014).

Since ancient times, plants have been utilized as harmless medicinal substances, treating certain infections

in both developed and underdeveloped countries (Batool *et al.*, 2018; Sharma *et al.*, 2009). Various herbs were used as preventative and curative traditional medicines against broad range of parasitic species (Al-Snafi, 2024). Secondary metabolites of plants are therefore frequently evaluated as alternative antibacterial agents. They are either directly used in form of precursors or as main compound in pharmaceuticals (Batool *et al.*, 2018; Sharma *et al.*, 2009). A variety of studies have reported activities of plant extracts for MDR strains (Batool *et al.*, 2018).

Some studies revealed that the combination of some plant extracts exhibited great inhibition properties in comparison to standard antibiotics. Combined effect of *Thymus vulgaris* and *Pimpinella anisum* has been observed against pathogenic bacteria (Al-Bayati, 2008). Combined antibacterial effects of ethanolic extracts from six medicinal plants were evaluated against gram positive and negative bacterial species, namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Karuncharoenpanich, 2025).

Coriandrum sativum (Coriander), *Murraya koenigii* (curry leave), and *Piper nigrum* (Black pepper) belong to aromatic herbs/ spices which are commonly used as flavouring food agents, have traditional medicinal aspects, and being frequently exploited for diabetes, inflammation, dentistry, as antipyretic agents, and other pharmacological attributes (Chaudhry and Tariq, 2006; Rautela *et al.*, 2018; Beegum *et al.*, 2019). In addition to that, their ethanolic, water and other extracts have reported antibacterial properties against, *S. aureus*, *S. typhi*, *K. pneumoniae*

(Beegum *et al.*, 2019; Matasyoh *et al.*, 2009). They also have reported antibacterial action against many MDR strains including *E. coli*, *M. luteus*, *S. aureus*, *P. aeruginosa* and *B. subtilis* (Vats *et al.*, 2011).

Antibacterial characteristics of *Coriandrum sativum*, *Murraya koenigii* and *Piper nigrum* have been reported in many studies, but their combination effects against uropathogenic bacteria have not been reported. In this study, coriander leaves, black pepper fruit, and curry leaves were extracted successively in n-hexane, ethyl acetate, methanol, distilled water. Their individual and combined inhibition actions were determined for selected uro-pathogenic ATCC bacterial strains along with MIC and MBC. Lastly, their TPC and TFC values were determined to get an idea about what compounds might be responsible for antibacterial effects of their extracts.

2. Materials and Methods

2.1. Plants Collection, Processing and Extraction

Spices/plants including *C. sativum*, *M. koenigii*, and *P. nigrum* were collected from local market, rinsed with normal water then distilled water, dried under shade, and grounded into fine powder. They were then subjected to successive solvent extraction (Jakhar *et al.*, 2015) with slight modifications (not utilizing Soxhlet apparatus) using solvents of different polarity namely n-hexane, ethyl acetate, methanol, and water as shown in Figure 1. Resultant extracts were filtered through muslin cloth and Whatman filter paper, rotary evaporated, and the extract efficiency was calculated.

2.2. Test Bacterial Strains

Four selected uropathogenic ATCC bacterial strains including *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923, *Acinetobacter baumannii* ATCC 45269, and *Streptococcus agalactiae* ATCC 9528 were collected National Veterinary Laboratory, and sub-cultured. Their antibiotic profile was evaluated by Kirby-Bauer's method (Bauer, 1966) and stored at 4°C in Nutrient broth (NB) for later use in extract antimicrobial evaluation.

2.3. Antibacterial Screening of Extracts

The method was adopted with slight modifications from Dash and co-workers (Dash *et al.*, 2017). Fifty μL inoculum (approx. $1-2 \times 10^8$ CFU/mL) (Ali *et al.*, 2017) was spread on solid Mueller Hinton agar (MHA) plates. Then, 5 μL of stock solution (20 mg/mL DMSO) containing paper discs (6 mm in diameter) were placed along with ceftriaxone/gentamycin as positive (20 μg /standard disc) and DMSO (5 μL soaked and dried on blank disc) as negative control. Plates were incubated for 24 hrs at 37°C, and the process was repeated for each of test strains in triplicates.

2.4. Combination Assay

Disk diffusion assay was used in triplicates for combination antibacterial assay as stated earlier (Dash *et al.*, 2017). Combinations were made in two different ways which were mixing equal ratio (1:1:1) i.e., 350 μL from three extracts for same solvent (20 mg/mL) stock solution together in Eppendorf tubes, whereas second combinations were made by mixing equal ratio (1:1) i.e., 500 μL of same solvent extracts together in Eppendorf tubes.

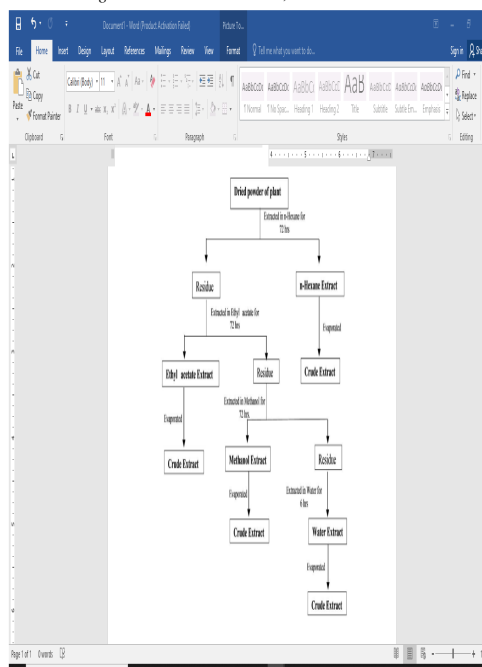


Figure 1: Schematic diagram of successive solvent extraction.

2.5. Minimum Inhibitory Concentration (MIC)

MICs determination method was adopted with slight modifications from Julianti and co-workers (Julianti *et al.*, 2017). Five μL of NB was added in each well leaving 1st row of microtiter plate vacant, where later 7.5 μL from sample wells were pipetted out and diluted 3-fold along length of the plate. The wells were inoculated with 195 μL strain culture and incubated for 24 hrs at 37°C. The process was repeated for all four test strains and positive control Ceftriaxone and Gentamycin. To determine MBCs, 20 μL from wells with no visible growth were streaked on MHA plates and incubated for 24 hrs at 37°C (Jeya *et al.*, 2019). The minimum concentrations that did not allow any growth were recorded as the minimum bactericidal concentration (MBC).

2.6. Total Phenolic and Flavonoid Content

The experiments were performed in Food Science Research Institute (FSRI) at National Agriculture Research Centre (NARC) Islamabad. Folin-Ciocalteu (FC) method (Do *et al.*, 2014) slightly modified was used for assessing Total Phenolic Content (TPC) with 2.5 mL of 7.5% sodium carbonate (Na_2CO_3) and 10% FC reagent, in which 0.5 mL of sample or diluted gallic acid standard was added. Absorbance was measured at 765 nm, calibration curve was constructed, and results were expressed as milligram gallic acid equivalent per gram (mgGAE/g) of extract. For Total Flavonoid Content (TFC) analysis, aluminium chloride colorimetric method described by Kamtekar and coworkers (Kamtekar *et al.*, 2014) was used with slight changes. One mL sample extract or quercetin standard was mixed with 4 mL dH_2O in which 0.3 mL of 5% sodium nitrite (NaNO_2) and 10% aluminium chloride (AlCl_3) was added. Volume was made up to 10 mL by 1M sodium hydroxide (NaOH). Absorbance was measured at 415 nm, calibration curve was prepared, and results displayed as milligram quercetin equivalent per gram (mg QCE/g) of extract.

2.7. Statistical Analysis

Microsoft excel pro 365 software was utilized to calculate mean and SEM. Every experiment was performed in triplicates in this study. To find difference among means for all extracts, One-way Analysis of Variance (ANOVA) was used. Results that have shown $P < 0.05$ were considered significant.

3. Results

Twelve extracts were obtained in semi solid forms except *P. nigrum* n-hexane (NP) which is obtained in oily form. Table 1 shows that maximum percentage yield/extract efficiencies (4.28%), was obtained for *P. nigrum* ethyl acetate (EP) extract among others, whereas *C. sativum* and *M. koenigii* have significant yields calculated for water extracts (WCO, 3.67%) and (WCU, 3.34%).

3.1. Antibacterial Activities

The results of antibacterial assays performed against all four test strains have revealed varying activities ($P < 0.05$). Figure 2 shows that overall, *A. baumannii* ATCC 45269 was the most effectively inhibited strain, with maximum inhibition zone (IZ) 10.33 ± 0.33 mm for WCo (water-coriander) extract.

Table 1. Percentage extract efficiency, total phenolic and total flavonoid content analysis \pm standard error mean of crude extracts.

S. No	Extract codes *	% Extract Yield	TPC mg GAE/g extract	TFC mg QE/g extract
1.	NCO	0.396 %	0.442 ± 0.005	0.217 ± 0.01
2.	ECO	1.35 %	1.41 ± 0.0	0.86 ± 0.0
3.	MCO	2.068 %	21.65 ± 0.66	9.994 ± 0.0
4.	WCO	3.67 %	3.55 ± 0.09	2.22 ± 0.0
5.	NP	1.47 %	5 ± 0.0	2.38 ± 0.03
6.	EP	4.28 %	7.292 ± 0.06	4.397 ± 0.0
7.	MP	1.34 %	29.36 ± 0.09	$15.048 \pm 0.0s$
8.	WP	1.43 %	31.16 ± 0.03	18.922 ± 0.03
9.	NCU	1.47 %	4.138 ± 0.01	1.933 ± 0.0
10.	ECU	2.01 %	6.278 ± 0.07	3.799 ± 0.02
11.	MCU	2.03 %	32.32 ± 0.06	17.563 ± 0.04
12.	WCU	3.34 %	28.72 ± 0.01	14.736 ± 0.05

*(NCO) n-hexane coriander, (ECO) ethyl acetate coriander, (MCO) methanol coriander, (WCO) distilled water coriander, (NP) n-hexane black pepper, (EP) ethyl acetate black pepper, (MP) methanol black pepper, (WP) water black pepper, (NCu) n-hexane curry leaves, (ECu) ethyl acetate curry, (MCu) methanol curry, (WCU) distilled water curry.

Inhibition activities of WCo against *S. aureus* and *E. coli* were measured to be 8.33 ± 0.33 mm and 8.00 ± 0.0 mm ($P < 0.05$), respectively. Likewise, *M. koenigii* extracts' most significant response was against *A. baumannii* with greatest IZ of 10.33 ± 0.0 mm for WCo (water-curry) extract, followed by *E. coli* (9.33 ± 0.33 mm), *S. aureus* (9.00 ± 0.0 mm) and *S. agalactiae* (9.00 ± 0.57 mm) IZs ($P < 0.05$), respectively (Table 2). In this study, *P. nigrum* antibacterial activity was greatly observed against *S. aureus* with IZ (10.33 ± 0.33 mm) for ethyl acetate (EP) extract ($P < 0.05$), followed by methanol (MP) extract against *A. baumannii* with IZ (10.00 ± 0.57 mm).

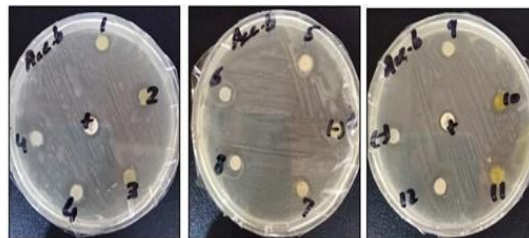


Figure 2: Individual inhibition action of extracts against *A. baumannii* ATCC 45269 strain.

3.2. Combination Activities

Table 3 summarizes the effective response exhibited individually by eight extracts made from combining them in (1:1) and (1:1:1) ratio against test strains. When *P. nigrum* and *M. koenigii* extracts evaluated in combinations (1:1), largest IZ was measured against *E. coli* (15 ± 0.57 mm) for WPCu (water-pepper-curry), followed by *A. baumannii* for EPCu (Ethyl acetate-pepper-curry), *S. aureus* for MPCu (methanol-pepper-curry) and *S. agalactiae* for NPCu (n-hexane-pepper-curry), respectively as shown in Figure 3. For other four combined extracts (1:1:1), *S. agalactiae* (12.66 ± 0.57 mm) and *E. coli* (12.33 ± 0.33 mm) showed significant IZ for ECoPCu (ethyl-acetate-coriander-pepper-curry), followed by *S. aureus* and *A. baumannii* (Figure 4).

3.3. Minimum Inhibitory Concentrations (MICs)

MIC and MBC values were determined for combined (1:1/1:1:1) extracts with IZs (> 10 mm) against test ATCC strains (Table 3). For (1:1) *M. koenigii* and *P. nigrum* combined extracts (WPCu), smallest MIC value of $12.5 \mu\text{g/mL}$ was recorded against *E. coli* with MBC of $25 \mu\text{g/mL}$ ($P < 0.05$).

Table 2. Growth inhibition activities in mm ± standard error mean exhibited by crude extracts against test bacterial strains.

S. No	Extracts * (20µg/mL)	Test Bacterial Strains			
		<i>S. aureus</i>	<i>E. coli</i>	<i>A. baumannii</i>	<i>S. agalactiae</i>
1.	NCO	7.00±0.57	6.00±0.0	7.66±0.33	6.00±0.0
2.	ECO	7.00±0.0	6.33±0.33	10.00±0.57	6.33±0.33
3.	MCO	6.33±0.33	7.00±0.0	8.00±0.57	6.00±0.0
4.	WCO	8.33±0.33	8.00±0.0	10.33±0.33	7.00±0.57
5.	NP	8.00±0.0	6.33±0.33	9.33±0.33	6.33±0.33
6.	EP	10.33±0.33	6.66±0.33	7.00±0.57	6.00±0.57
7.	MP	9.00±0.0	9±0.57	10.00±0.57	7.00±0.0
8.	WP	8.67±0.33	8.33±0.33	7.66±0.33	9.00±0.57
9.	NCU	8.00±0.57	6.66±0.33	9.00±0.57	10.00±0.0
10.	ECU	8.00±0.00	8.33±0.33	9.00±0.57	7.00±0.57
11.	MCU	8.00±0.57	8.00±0.0	8.00±0.0	7.66±0.33
12.	WCU	9.00±0.0	9.33±0.33	10.33±0.0	9.00±0.57
Positive control	Gentamicin	16.33±0.66	† ___	___	19.66±0.33
	Ceftriaxone	___	15.33±0.33	8.33±0.33	___
Negative control	‡ DMSO	§ NIZ	NIZ	NIZ	NIZ

*(NCo) n-hexane coriander, (ECO) ethyl acetate coriander, (MCo) methanol coriander, (WCo) distilled water coriander, (NP) n-hexane black pepper, (EP) ethyl acetate black pepper, (MP) methanol black pepper, (WP) water black pepper, (NCu) n-hexane curry leaves, (ECu) ethyl acetate curry, (MCu) methanol curry, (WCU) distilled water curry. † (-) - Not determined, ‡ (DMSO) - Dimethyl sulphoxide, § (NIZ) - No inhibition zone.

Table 3. Inhibition activities ± standard error means combination extracts with their minimum inhibitory concentration and minimum bactericidal concentrations against test strains. All MIC and MBC values were taken in triplicate.

S. No	Test Strains	Extracts *	IZ (mm± SEM)	MIC (µg/mL)	MBC (µg/mL)
1.	<i>A. baumannii</i> ATCC 45269	ECuPCo	10.66±0.33	25	50
		WCuPCo	11.66±0.33	25	100
		NPCu	10±0.57	50	100
		EPCu	13.33±0.33	25	50
		MPCu	10±0.57	50	100
		WPCu	12±0.57	25	
		Control	8.33±0.33		
		ECuPCo	12.33±0.33	50	100
		MCuPCo	11.33±0.66	50	100
		2.	<i>E. coli</i> ATCC 8739	NPCu	12±0.57
WPCu	15±0.57			12.5	25
Control	15.33±0.33			12.5	
NCuPCo	11.33±0.33			100	100
ECuPCo	11.33±0.33			100	100
3.	<i>S. aureus</i> ATCC 25923	EPCu	12±0.57	100	100
		MPCu	12.33±0.66	50	50
		WPCu	13±0.57	50	50
		Control	16.33±0.66	6.25	
		NCuPCo	11±0.57	50	50
4.	<i>S. agalactiae</i> ATCC 9528	ECuPCo	12.66±0.33	25	50
		WCuPCo	11±0.57	50	100
		NPCu	12.33±0.33	50	100
		MPCu	12±0.57	50	100
		Control	19.66±0.33	6.25	

*(NCoPCu) n-hexane coriander-pepper-curry, (ECOPCu) ethyl acetate coriander-pepper-curry, (MCoPCu) methanol coriander-pepper-curry, (WCoPCu) distilled water coriander-pepper-curry, (NP-Cu) n-hexane black pepper-curry, (EP-Cu) ethyl acetate pepper-curry, (MP-Cu) methanol pepper-curry, (WP-Cu) distilled water pepper-curry.

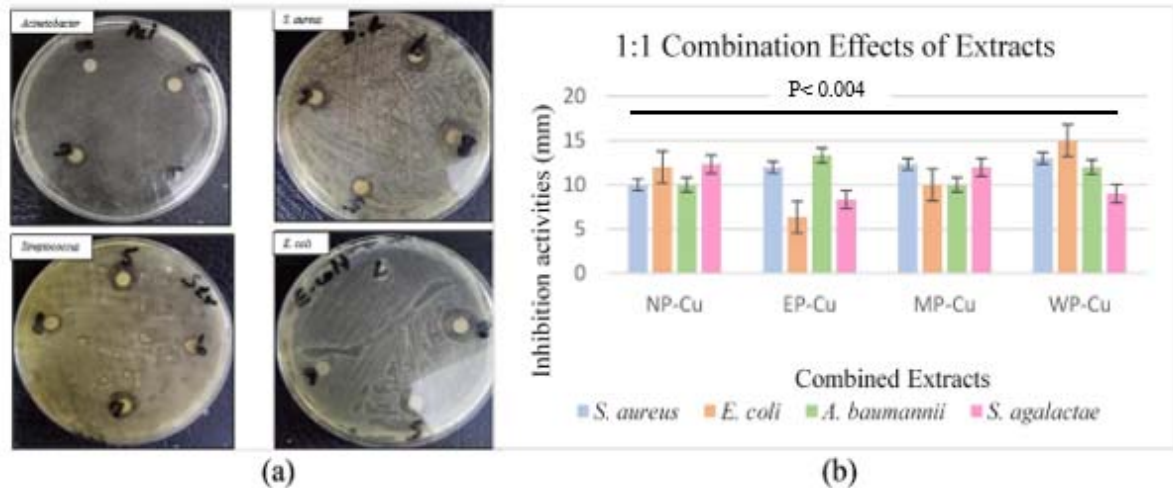


Figure 3: Inhibition action of (1:1) combined extracts (a) Inhibition zone for combined extracts against test ATCC strains on MHA agar plates; (b) Their comparative effects in graphical form.

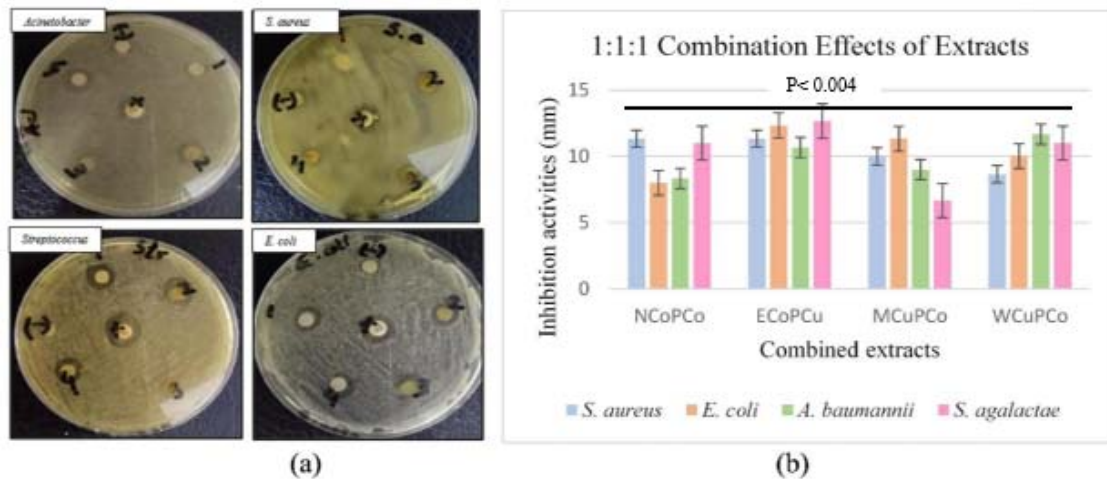


Figure 4: Inhibition action of (1:1:1) combined extracts (a) Inhibition zone for combined extracts against test ATCC strains on MHA agar plates; (b) Their comparative effects in graphical form.

Whereas MIC for (1:1:1) combination extracts of *C. sativum*, *M. koenigii* and *P. nigrum* was determined 25 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ ($P < 0.05$) against *A. baumannii* and *S. agalactae*, for both WCuPCo (water-curry-pepper-coriander) and ECoPCo extracts, MCuPCo (metabolic-curry-pepper-coriander) extract has shown 50 $\mu\text{g/mL}$ MIC for *E. coli*. MBCs were recorded by inoculating broth from least turbid wells of microtiter plate on agar media.

3.4. Total Phenolic and Flavonoid Content

From the values of TPC and TFC for obtained extract (Table 1), it follows that among all extracts, largest TPC/TFC values were observed for methanolic and dH₂O extracts, i.e. MCo, MP, MCu, WCo, WP and WCu of *C. sativum*, *M. koenigii* and *P. nigrum*, as shown in Figure 5. *P. nigrum* (MP and WP) extracts had TPCs of 29.36 and 31.16 mg GAE/g extracts.

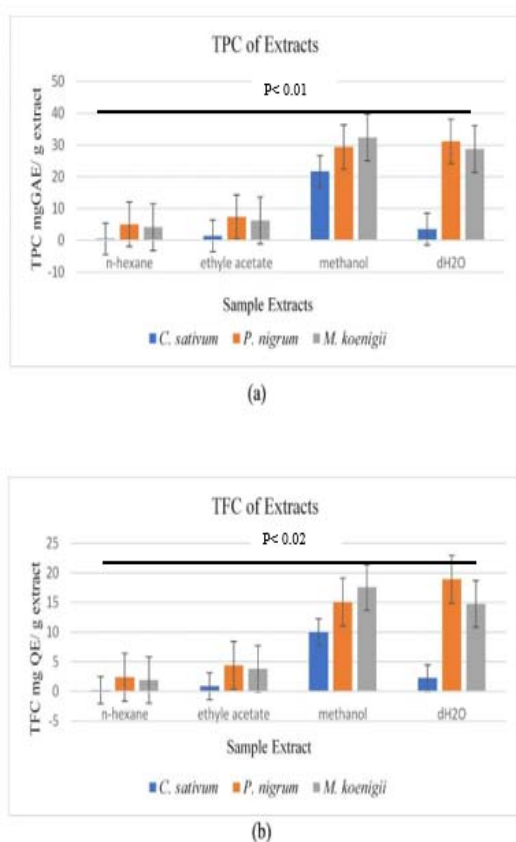


Figure 5: (a) Comparative TPCs expressed as mgGAE/ g extract of all sample extracts in graphical form; (b) Comparative TFCs expressed as mg QE/ g extract of all sample extracts in graphical form.

On the other hand, their observed TFCs were recorded to be 15.048 and 18.922 mg/g of dry weight and expressed as quercetin equivalent ($P < 0.05$) respectively in this study. The observed TPCs of *C. sativum* (MC₀) were 21.65 mg GAE/ g extract, whereas its TFC was measured to be 9.994 mg QE/ g of extract ($P < 0.05$) (Figure 5).

4. Discussion

The values for extract yield calculated (WCO, 3.67% and WCU, 3.34%) were in accordance to that stated by Jeya *et al.* (2019) and Hussain *et al.* (2009), where reported yield for *C. sativum* seed EOs ranged from 0.03%-2.6%. Caleja *et al.* (2016) reported that water extracts have more yield than ethanol extracts as observed in this study for *C. sativum* and *M. koenigii*. The reason for this might be high H₂O polarity (Dhanani *et al.*, 2017) or use of leaves or fruiting parts of *C. sativum*, *M. koenigii*, and *P. nigrum* respectively, for extract preparation.

Rezaei *et al.* (2016) reported antibacterial action of *C. sativum* leaves EO with IZs of about 12.5 mm and 8.5 mm against *S. aureus* and *E. coli*. Water extracts of *M. koenigii* had observed IZs of 8.33 ± 0.33 mm and 8.00 ± 0.00 mm against *E. coli* and *S. aureus* respectively (Razak, 2020) as reported in this study. Nagy *et al.* reported a 10.5 mm IZ, whereas Khan *et al.* observed no inhibition for the methanolic *P. nigrum* extract against *S. aureus* (Khan *et al.*, 2013; Nagy *et al.*, 2015). These values contrasted either greatly or a little with IZs observed in the current study.

There was no literature available on combined effects of *P. nigrum*, *M. koenigii* and *C. sativum*, yet their combinations were tested with standard antimicrobials or other plant's EOs, where *M. koenigii* aqueous extract (1:1) combination with *Telfairia occidentalis* (fluted pumpkin) have exhibited 31.25mg/mL MIC and 250mg/mL MBC values for both *E. coli* and *S. aureus* respectively (Akinribosun and Ogu, 2017). Antibacterial activities of methanolic extracts of *P. nigrum* in combination with standard antibiotics (ampicillin, gentamicin and erythromycin among others) against different strains of *E. coli* were observed with MICs (2-16 μ g/mL) (El-Tawab *et al.*, 2018).

In this study, the MIC and MBC values of 1:1 combined extracts of *M. koenigii* and *P. nigrum* with IZs (> 10 mm) against test ATCC strains of *E. coli* and *S. aureus* were determined. The smallest MIC value measured to be was 12.5 μ g/mL for aqueous extract (WPCu) against *E. coli* with MBC of 25 μ g/mL. MIC and MBC values for *S. aureus* of aqueous and methanolic extracts (WPCu, MPCu) were 50 μ g/mL. In contrast to these, MIC values of 40 μ g/mL, 80 μ g/mL and 100 μ g/mL were observed for co-trimoxazole against various isolates of UTI, primarily *E. coli*, *Klebsiella* and *Pseudomonas* respectively (Qadir *et al.*, 2024).

As reported in this study, the potential of synergistic effects of medicinal plant *Calotropis procera* extract was also observed with amoxicillin, ampicillin and azithromycin antibiotics against *S. aureus*, *E. coli* and *Shigella spp.* with IZ of (16.8 mm, 21.33 mm and 16.4 mm) respectively (Gideon and Lada, 2023). In another study, Khunbutsri *et al.* (2022) reported that leaf extract of *Solanum torvum* exhibited MIC and MBC of about 16mg/mL and 18mg/mL respectively against methicillin resistant *Staphylococci*. When the said extract is evaluated in combination with oxacillin antibiotic, it has shown synergistic, partial synergistic as well additive effects against *S. epidermidis* and *S. intermedius* (Khunbutsri *et al.*, 2022).

As reported by Ahmed *et al.*, observed TPC of methanolic *P. nigrum* extract was 1.7281 mg/g dry weight and expressed as GAE, whereas TFC value was measured to be 1.087 μ g/g quercetin equivalent (Ahmad *et al.*, 2015). TPC of 17.04 mg GAE/ 100 g dry weight was observed, with TFC of 11.10 mg/ 100 g dry weight and expressed as catechin equivalent for methanolic extract of *C. sativum* (Christova-Bagdassaria *et al.*, 2013). TPC and TFC for methanolic curry leaves extracts were 13.68 mg GAE/ g fresh weight and 0.81 mg CE/g fresh weight respectively (Hijaz *et al.*, 2020). Lastly, TPC values for (MCu and WCu) *M. koenigii* were observed about 32.32 and 19.57 mg GAE/ g of extracts with their TFCs of 17.56 and 11.73 mg QE/g extract respectively. The results suggested that said plants possess potential attributes when used in combinations and could be useful for future medicinal formulations for resistant uro-pathogens.

5. Conclusion

Present study reveals effectiveness of three antimicrobial plant sources, i.e. *Coriandrum sativum*, *Murraya koenigii* and *Piper nigrum*. In combination, they show marked inhibition of uropathogenic bacterial strains. Hence, they could be useful for future medicinal formulations for resistant uro-pathogens. Moreover, this study paves ways for research on natural antimicrobials to be used in combinations for effective response against pathogenic bacteria which are speedily becoming resistant.

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References

- Ahmad A, Husain A, Mujeeb M, Khan SA, Alhadrami HAA, and Bhandari A. 2015. Quantification of total phenol, flavonoid content and pharmacognostical evaluation including HPTLC fingerprinting for the standardization of *Piper nigrum* Linn fruits. *Asian Pac. J. Trop. Biomed*, **5(2)**: 101-107.
- Ahmad S, Hussain A, Khan MSA, Shakireen N, and Ali I. 2020. Diabetes mellitus and urinary tract infection: Causative uropathogens, their antibiotic susceptibility pattern and the effects of glycemic status. *Pak J Med Sci*, **36(7)**: 1550.
- Akinnibosun F, and Ogu GI. 2017. Antibacterial Activity of Aqueous and Ethanolic Leaf Extracts of *Murraya koenigii* and *Telfairia occidentalis* Synergy. *NJAFE*, **13(1)**: 234-239.
- Al-Bayati FA. 2008. Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts. *J. Ethnopharmacol*, **116(3)**: 403-406.
- Ali ST, Ayub A, and Ali SN. 2017. Antibacterial activity of methanolic extracts from some selected medicinal plants. *FUUAST j. biol*, **7(1)**: 123-125.
- Al-Snafi AE. 2024. Antiparasitic activities of medicinal plants: An overview. *GSC Biol. Pharm. Sci*, **27(2)**: 167-223.
- Batool K, Sultana S, Akhtar N, Asif HM, Akhtar N, Ahmad K, and Owais A. 2018. Medicinal plants combating against human pathogens: A review. *J. Int. J. Biotechnol. Food Sci*, **6(3)**: 42-51.
- Bauer AW. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol*, **45**: 149-158.
- Beegum N, Reshmi R, Nandan N, and Vishwanathan S. 2019. Spices-An imperative melange-back to the roots. *J Ayurveda Integr Med*, **4(6)**: 93-103.
- Caleja C, Barros L, Antonio AL, Carochi M, Oliveira MBP, and Ferreira IC. 2016. Fortification of yogurts with different antioxidant preservatives: A comparative study between natural and synthetic additives. *Food Chem*, **210**: 262-268.
- Chen YH, Ko WC, and Hsueh PR. 2013. Emerging resistance problems and future perspectives in pharmacotherapy for complicated urinary tract infections. *Expert Opin Pharmacother*, **14(5)**: 587-596.
- Chaudhry NMA, and Tariq P. 2006. Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pak. J. Pharm. Sci*, **19(3)**: 214-8.
- Christova-Bagdassarian VL, Bagdassarian KS, and Atanassova MS. 2013. Phenolic profile, antioxidant and antimicrobial activities from the Apiaceae family (dry seeds). *MJPMS*, **2(4)**: 26-31.
- Dash GK, Sekar M, Adiba SSP, and Mahmud A. 2017. Antibacterial activity of *Murraya koenigii* against few *Staphylococcus* spp. and development of a topical cream. *Indo Am. j. pharm. sci*, **4(9)**: 2976-2980.
- Dhanani T, Shah S, Gajbhiye NA, and Kumar S. 2017. Effect of extraction methods on yield, phytochemical constituents, and antioxidant activity of *Withania somnifera*. *Arab. J. Chem*, **10**: S1193-S1199.
- Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, and Ju YH. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *J. Food Drug Anal*, **22(3)**: 296-302.
- El-Tawab A, Selim AO, and Soliman AM. 2018. Phenotypic and Genotypic Characterization of Some Bacterial Isolates (*Escherichia Coli*, *Klebsiella Oxytoca*) From Chickens. *Benha Vet. Med. J*, **35(2)**: 284-302.
- Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, and Nakhband A. 2009. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. *Int. J. Infect. Dis*, **13(2)**: 140-144.
- Gideon M, and Lada Z. 2023. Synergistic combinatorial strategy for combating Antimicrobial Resistance (AMR) in clinical bacteria by combining antibiotics with plant extracts. *FCE*, 1-12.
- Gupta K, and Bhadelia N. 2014. Management of urinary tract infections from multidrug-resistant organisms. *Infect Dis Clin North Am*, **28(1)**: 49-59.
- Hijaz F, Al-Rimawi F, Manthey JA, and Killiny N. 2020. Phenolics, flavonoids and antioxidant capacities in Citrus species with different degree of tolerance to Huanglongbing. *Plant Signal Behav*, **15(5)**: 1752447.
- Hussain J, Khan AL, Rehman N, Zainullah KF, Hussain ST, and Shinwari ZK. 2009. Proximate and nutrient investigations of selected medicinal plants species of Pakistan. *Pak J Nutr*, **8(5)**: 620-624.
- Jakhar S, Gahlawat DK, Dahiya S, Swami U, Verma M, and Dahiya P. 2015. Antibacterial and Antioxidant Potential of Leaf and Seed Extracts of *Murraya koenigii* (Linn.) Spreng. *Microbiol. Res. J. Int*, 1-7.
- Jeya KR, Veerapagu M, and Sangeetha V. 2019. Antimicrobial and antioxidant properties of *Coriandrum sativum* L. seed essential oil. *Am. J. Essent. Oil. Nat. Prod*, **7(2)**: 06-10.
- Julianti E, Rajah KK, and Fidrianny I. 2017. Antibacterial activity of ethanolic extract of cinnamon bark, honey, and their combination effects against acne-causing bacteria. *Sci. Pharm*, **85(2)**: 19.
- Kamtekar S, Keer V, and Patil V. 2014. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. *J. Appl. Pharm. Sci*, **4(9)**: 61.
- Karuncharoenpanich T, Phetmanee T, Pradubay N, Songsak T, Jongrungruangchok S, Madaka F, and Lakkana N. 2025. In Vitro Assessment of Antimicrobial Activity and Synergistic Effects of Ethanolic Extracts from Six Medicinal Plants. *J. Curr. Sci. Technol*, **115(1)**: 85-85.
- Kowalska-Krochmal B, and Dudek-Wicher R. 2021. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, **10(2)**: 165.
- Khan AU, Ali S, Rehman AU, Ali H, Ahmad T, Waqar M, and Niaz Z. 2013. Antibacterial Activity of *Nigella sativa* and *Piper nigrum*. *AJSC*, **2(4)**: 173-9.
- Khunbutsri D, Naimon N, Satchasataporn K, Inthong N, Kaewmongkol S, Sutjarit S, and Meekhanon, N. 2022. Antibacterial activity of *Solanum torvum* leaf extract and its synergistic effect with oxacillin against methicillin-resistant *Staphylococci* isolated from dogs. *Antibiotics*, **11(3)**: 302.
- Matasyoh JC, Maiyo ZC, Ngure RM, and Chepkorir R. 2009. Chemical composition and antimicrobial activity of the essential oil of *Coriandrum sativum*. *Food Chem*, **113(2)**: 526-529.
- Mirsoleymani SR, Salimi M, Shareghi Brojeni M, Ranjbar M, and Mehtarpoor M. 2014. Bacterial pathogens and antimicrobial resistance patterns in paediatric urinary tract infections: a four-year surveillance study (2009–2012). *Int. J. pediatr*, 2014.
- Nagy M, Socaci SA, Tofana M, Pop C, Muresan C, Pop AV, and Rotar A. 2015. Determination of total phenolics, antioxidant capacity and antimicrobial activity of selected aromatic spices. *Bulletin UASVM Food Science and Technology*, **72(1)**: 82-85.

- Paterson DL. 2006. Resistance in gram-negative bacteria: *Enterobacteriaceae*. *Am J Infect Control*, **34(5)**: S20-S28.
- Qadir H, Rehman MA, Nasir S, Alam MA, Ibrar M, and Shuaib SL. 2024. Evaluation of Antibiotics by Disk Diffusion and Minimum Inhibitory Concentration Breakpoints in Urinary Tract Infections: Evaluation of Antibiotics for Urinary Tract Infections. *PJHSL*, 183-186.
- Rautela R, Das GK, Khan FA, Prasad S, Kumar A, Prasad JK, and Srivastava SK. 2018. Antibacterial, anti-inflammatory and antioxidant effects of *Aegle marmelos* and *Murraya koenigii* in dairy cows with endometritis. *Livest. Sci*, **214**: 142-148.
- Razak WRWA. 2020. Antimicrobial activity of curry leaves (*Murraya koenigii*) on selected foodborne pathogens. *Sci. Lett*, **14(1)**: 7-13.
- Rezaei M, Karimi F, Shariatifar N, Mohammadpourfard I, and Malekabad ES. 2016. Antimicrobial Activity of the Essential Oil from the Leaves and Seeds of *Coriandrum sativum* toward Foodborne Pathogens. *West Indian Med. J*, **65(1)**.
- Sharma A, Verma R, and Ramteke, P. 2009. Antibacterial Activity of Some Medicinal Plants Used by Tribals Against UTI Causing Pathogens. *World Appl. Sci. J*. **7(3)**: 332-339.
- Singh SD, and Madhup SK. 2013. Clinical profile and antibiotics sensitivity in childhood urinary tract infection at Dhulikhel Hospital. *Kathmandu Univ Med J*, **11(4)**: 319-324.
- Vats M, Singh H, and Sardana S. 2011. Phytochemical screening and antimicrobial activity of roots of *Murraya koenigii* (Linn.) Spreng. (*Rutaceae*). *Braz J Microbiol*, **42(4)**: 1569-1573.