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Antioxidant and Antimicrobial Capacities of Two Medicinal Plants Used against Urinary Infections in Burkina Faso

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Abstract

Objective: This study aimed to evaluate the antioxidant and antimicrobial capacities of extracts from *Euphorbia hirta* L. and *Terminalia avicennioides* GUILL & PERR. **Methodology:** The crude hydro-acetonic and aqueous extracts as well their fractionations were prepared. The total phenolic, flavonoids and tannins contents were assessed using the Folin-Ciocalteu, aluminum chloride and vanillin acid methods, respectively. The antioxidant and antibacterial activities were investigated using standard methods. **Results:** *Euphorbia hirta* showed significant contents of total phenolic and flavonoids in n-Butanol (145.14 ± 1.37 GAE/100mg extracts) and ethyl acetate (23.56 ± 0.68 mg QE/100mg extracts) fractions. Total tannins were high in hydro-acétonique extract (11.18 ± 0.31 mg TAE/100mg extracts) and aqueous fraction (11.12 ± 0.28 mg TAE/100mg extracts) of *Terminalia avicennioides* stem barks. Extracts and fractions of both plants demonstrated a strongly antioxidant capacity through the free radicals scavenging and the ferric ions reducing. Concerning antimicrobial screening the extracts of *Terminalia avicennioides* were effective against 16 causative pathogens of urinary tract infections. Bactericidal effect against *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* and 2 methicillin-resistant *Staphylococcus haemolyticus* strains was found with aqueous fraction of *Terminalia avicennioides* leaves. This fraction also highlighted a synergetic effect with some antibiotics used against these bacterial strains. **Conclusion:** *Terminalia avicennioides* leaves could be recommended as an

herbal drug formulation for the urinary infections management.

Keywords

Antimicrobial, Antioxidant, *Euphorbia hirta* L., *Terminalia avicennioides* GUILL & PERR, Urinary Infections

1. Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections [1]. Indeed, UTIs affect approximately 250 million people annually worldwide, and are the main cause of medical visits which impose a substantial financial burden on society [2]. Antibiotic therapies remain the most effective approach for the management of UTIs [3].

Unfortunately, many therapeutic failures are observed because of the abusive and inappropriate uses of antibiotics [4]. Recently there is a resurgence of interest in phytochemicals, to search for new safe potential inhibitors, due to the emergence of multi-antibiotic-resistant pathogens [5]. Phenolic compounds (PCs) are among the most phytochemicals explored because they have enormous scope of biological effects [6].

PCs include classes of molecules known as tannins, lignins, and flavonoids. That exhibited various physiological activities, including anti-inflammatory, antioxidant, anti-carcinogenic, antihypertensive, anti-arthritic, and antimicrobial [7]. PCs could be also a good supplement during antibiotic therapy that may accelerate the antibacterial action, as well as inhibiting oxidative damages generated by the action of antibacterial agents [5].

Numerous ethnobotanical surveys carried out worldwide have revealed that various medicinal plants are commonly used to treat UTIs [8] [9] [10]. *Euphorbia hirta* L. (*E. hirta*) and *Terminalia avicennioides* GUILL & PERR (*T. avicennioides*) are two plants frequently used in the management of UTIs in the Hauts-Bassins areas of Burkina Faso. Those plants are widely applied as hypoglycemic, antimicrobial and they are used for treating inflammatory diseases, parasitosis, cough and rheumatism [11]. The aim of this study was to investigate the antioxidant and antibacterial effects of extracts and fraction from *E. hirta* and *T. avicennioides*.

2. Materials and Methods

2.1. Standards and Reagents

The Folin-Ciocalteu reagent, NaH_2PO_4, Na_2HPO_4, sodium carbonate, aluminum trichloride, gallic acid, tannic acid and quercetin were purchased from Sigma-aldrich chemie, Germany. 2,2-diphenylpicrylhydrazyl (DPPH), trichloroacetic acid, and solvents used were from Fluka Chemie, Switzerland. Potassium hexacyanoferrate [$K_3Fe(CN)_6$] was from Prolabo and ascorbic acid was from Labosi, France.

All chemicals and solvents used were of analytical grade.

2.2. Plant Collection

The leaves stem barks, roots of *T. avicennioides* and the whole plant of *E. hirta* were collected during the month of May 2022 from the classified forest of Dinderesso, a village located about 15 km west of Bobo-Dioulassso/Burkina Faso. The plants were identified by Dr Ouaba Yempabou Hermann a botanist-cytoecologist from the University NAZI Boni of Bobo-Dioulasso. The organs of plants collected were washed, dried in the shade and then powdered for further analysis.

2.3. Microbial Strains

Standard reference strains, including *Escherichia coli* (ATCC 25922), *Escherichia coli* (ATCC 35218), *Klebsiella pneumoniae* (ATCC 700603) and *Staphylococcus aureus* (ATCC 29213). Clinical strains from suspected infected with UTIs patients, including *Staphylococcus aureus*, *Klebsiella pneumoniae* (n = 4), *Escherichia coli* (n = 11), *Enterobacter aeroginosa*, *Acinetobacter baumannii* (n = 2), *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus haemolyticus* (n = 2), *Streptococcus agalactiae* and *Candida albicans* (n = 3). All the strains were obtained from the bacteriology research laboratory of the Muraz center/Burkina Faso. A previous antibiogram identified bacterial isolates of ESBL (4 *K. pneumoniae* et 2 *E. coli*) and methicillin-resistant (2 *Staphylococcus haemolyticus*) phenotypes.

2.4. Preparation of Extracts

Decoction: 25 g of each plant powder were extracted with 250 ml of distilled water. The mixture was heated and boiled under reflux at 100˚C for 30 min. After filtration, the extracts were frozen and lyophilized for obtaining the aqueous crude extract.

Maceration: 25 g of each powdered plant samples were extracted with aqueous acetone (80%). After 24 hours of mechanical stirring at room temperature, the acetone was removed using a rotary evaporator at 60˚C, and the rest was lyophilized for corresponding to hydro-acetonic crude extract.

Fractionation: crude extracts was made by successive liquid-liquid fractionation with an equal volume of n-Hexane, Dichloromethane, Ethyl acetate and n-Butanol. The extracts were concentrated to dryness and stored at 4˚C until being used.

2.5. Phenolic Compounds Content

Total Phenolic and total flavonoids: Total phenolic and total flavonoids were assessed using the Folin-Ciocalteu and aluminum chloride reagents, respectively as described previously [12]. The results were expressed in mgEAG/100mg and mgEQ/100mg extract, respectively.

Total Tannins: Tannin estimation was done by treating extracts with vanillin

acid [13]. The results were expressed in mgTAE/100mg extract.

2.6. Evaluation of Antioxidant Properties

The free radical (DPPH) scavenging capacity and the ferric reducing power (FRAP) of extracts and fractions of both plants were evaluated as described previously by other author [12].

2.7. Antimicrobial Testing

Antimicrobial activities of plant extracts were carried out by applying the disc diffusion method [14]. The evaluation of the efficacy of the extracts was made according to the criteria from a previous study [15].

2.7.1. Determination of Antibacterial Parameters

Minimum Inhibitory Concentration (MIC): The MIC values were determined using a broth microdilution assay as described earlier [16]. The lowest concentration of extract required for inhibiting the growth of bacteria was recorded as the MIC.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC): The MBC/MBF was determined from the positive MIC well, according to other study [17]. The MBC/MBF value was estimated as the lowest concentration of extract that inhibits 98% - 99.9% of microbes.

The MBC/MIC ratio defines the mode of action of the substance. The effect is bactericidal if the ratio is below 4, bacteriostatic if it is over 4 or tolerant if it exceeds 32 [18].

2.7.2. Synergy Assay

The double disc method was used to explore the synergistic action between antibiotic discs with an extract. Antibiotic discs uniquely and the disc combination with 20 µl of extract were put apart on a Mueller-Hinton agar plate which was inoculated with pathogens of 0.5 McFarland turbidity. After 24 h incubation at 37˚C, a positive interaction is suggested by the enlargement of the size of inhibition zones [19].

2.8. Statistical Analysis

The analysis was performed in triplicate and the results were expressed as mean value ± standard error. The direction and the magnitude of the correlation between the variables were calculated using analysis of variance (ANOVA test) and Pearson's correlation test (r^2). The criterion for statistical significance was $p \leq 0.05$.

3. Results

3.1. Polyphenols Content

Variable contents in phenolics, flavonoids and total tannins from crude extracts and fractions of *E. hirta* and *T. avicennioides* are shown in **Table 1**. The n-Butanol

Table 1. Phenolics contents of *E. hirta* and *T. avicennioides* extracts.

Plants	Organs	Extracts/Fractions	Total phenolics (mgEAG/100mg)		Total Flavonoids (mgEQ/100mg)		Total Tannins (mgEAT/100mg)	
			Maceration	Decoction	Maceration	Decoction	Maceration	Decoction
E. hirta	Whole plant	Crude	45.95 ± 0.76	18.74 ± 0.45	5.96 ± 0.28	0.71 ± 0.21	1.39 ± 0.02	0.23 ± 0.01
		n-Hexane	7.82 ± 0.45	11.99 ± 0.61	2.15 ± 0	0.16 ± 0	2.14 ± 0.10	0.28 ± 0
		Dichloromethane	13.39 ± 0.45	14.14 ± 1.21	4.09 ± 0.17	0.47 ± 0.2	2.06 ± 0.13	0.15 ± 0.12
		Ethyl acetate	49.7 ± 0.91	36.63 ± 4.54	23.56 ± 0.68[a]	14.03 ± 0.17	0.47 ± 0.03	2.12 ± 0.1
		n-Butanol	43.20 ± 0.76	145.14 ± 1.37[a]	6.79 ± 0.28	18.32 ± 0.17[a]	0.44 ± 0.03	1.55 ± 0.11
		Aqueous	21.74 ± 1.67	14.67 ± 1.06	1.67 ± 0.16	0.66 ± 0.33	0.63 ± 0.06	0.11 ± 0.01
T. avicennioides	leaves	Crude	60.09 ± 1.06	40.92 ± 1.75	4.05 ± 0.08	3.26 ± 0.14	4.66 ± 0.07	1.03 ± 0
		n-Hexane	17.14 ± 0.61	24.42 ± 1.21	1.376 ± 0.23	2.25 ± 0.12	2.25 ± 0.08	0.35 ± 0.07
		Dichloromethane	10.14 ± 0.89	9.43 ± 0.30	1.51 ± 0.32	0.9 ± 0.09	8.71 ± 0	0.27 ± 0.04
		Ethyl acetate	58.16 ± 0.45	67.62 ± 0.45	4.567 ± 0.17	4.10 ± 0.09	3.16 ± 0.16	2.11 ± 0.27
		n-Butanol	46.92 ± 0	68.02 ± 0.15	4.47 ± 0.36	6.43 ± 0.08	3.16 ± 0.15	1.66 ± 0.10
		Aqueous	40.49 ± 0.30	33.99 ± 0.87	3.24 ± 0.11	1.62 ± 0.05	1.27 ± 0.09	1.71 ± 0.14
	Stem barks	Crude	56.02 ± 0.15	59.66 ± 1.06	4.95 ± 0.12	4.82 ± 0.18	11.18 ± 0.31[a]	6.33 ± 0.17
		n-Hexane	20.46 ± 0.76	46.17 ± 0.45	0.741 ± 0.05	3.31 ± 0.3	3.32 ± 0.05	6.98 ± 0.08
		Dichloromethane	10.60 ± 0.17	68.84 ± 1.39	3.892 ± 0.21	3.23 ± 0.17	7.29 ± 0.27	1.81 ± 0.11
		Ethyl acetate	61.03 ± 2.39	101.76 ± 1.5	8.10 ± 0.11	6.01 ± 0.05	8.65 ± 0.93	6.39 ± 0.07
		n-Butanol	64.05 ± 2.42	79.13 ± 0.32	0.60 ± 0.06	7.36 ± 0.12	7.95 ± 0.61	10.60 ± 0.45[a]
		Aqueous	81.41 ± 0.91[a]	58.06 ± 1.11	4.87 ± 0.17	2.20 ± 0.05	11.12 ± 0.28[a]	10.96 ± 0.08[a]
	Roots	Crude	64.91 ± 0.9	39.63 ± 0.30	9.81 ± 0.14	2.01 ± 0.06	4.21 ± 0.16	1.40 ± 0
		n-Hexane	9.21 ± 0.74	23.78 ± 1.5	0.29 ± 0.02	4.85 ± 0.13	3.06 ± 0.28	0.35 ± 0.04
		Dichloromethane	14.50 ± 0.99	29.99 ± 1.34	0.62 ± 0.01	4.24 ± 0.05	1.11 ± 0.1	1.31 ± 0
		Ethyl acetate	46.59 ± 1.67	60.30 ± 0.15	2.54 ± 0.11	8.29 ± 0.05	2.52 ± 0.12	2.94 ± 0.07
		n-Butanol	66.73 ± 0.45	78.513 ± 1.36	7.2 ± 0.38	7.55 ±.0	3.82 ± 0.42	2.78 ± 0.06
		Aqueous	68.55 ± 3.33	42.20 ± 1.21	9.66 ± 0.53	4.87 ± 0.23	1.80 ± 0.05	1.61 ± 0.71

Values with superscript letters a mean highest content.

fraction (145.14 ± 1.37 GAE/100mg extracts) from *E. hirta* and the Aqueous fraction of stem barks from *T. avicennioides* (81.41 ± 0.91 mg GAE/100mg extracts) had high levels of phenolic. For total flavonoids, the best contents (23.56 ± 0.68 and 18.32 ± 0.17 mg QE/100mg extracts) were obtained with ethyl acetate and n-Butanol fractions of *E. hirta*, respectively. Total tannins were high in Aqueous acetone extract (11.18 ± 0.31 mg TAE/100mg extracts) as well as Aqueous fraction (11.12 ± 0.28 mg TAE/100mg extracts) and n-Butanol fraction (10.60 ± 0.45 mg TAE/100mg extracts) of *T. avicennioides* stem barks.

3.2. Antioxidant Capacity

Free radical scavenging (DPPH) and Ferric Reducing Antioxidant Power (FRAP) methods have been used to measure the antioxidant activity of *E. hirta* and *T. avicennioides* extracts (Table 2). In DPPH assay the results ranged from 39.14 ± 8.95 µmol AAE/g extract (n-Hexane fraction of *E. hirta*) to 920.06 ± 41.28 µmol AAE/g extract (Ethyl Acetate fraction of *T. avicennioides* roots). Concerning the ferric reducing capacity of extracts, the values varied between 373.37 ± 12.29 µmol AAE/g extract (Dichloromethane fraction of *T. avicennioides* leaves) and 12399.21 ± 196.75 µmol AAE/g extract (n-Butanol fraction of *E. hirta*).

Table 2. Antioxidant activity of *E. hirta* and *T. avicennioides* extracts.

Plants	Organs	Extracts/ Fractions	DPPH µmolEAA/g extract		FRAP µmolEAA/g extract	
			Maceration	Decoction	Maceration	Decoction
E. hirta	Whole plant	Crude	657.81 ± 1.67	405.96 ± 9.21	3507.89 ± 24.57	1085.35 ± 36.86
		n-Hexane	238.33 ± 7.81	39.14 ± 8.95	547.02 ± 12.28	442.82 ± 12.29
		Dichloromethane	366.41 ± 1.81	123.82 ± 9.03	422.54 ±10.03	659.91 ± 24.54
		Ethyl acetate	850.26 ± 27.64	801.76 ± 2.56	3282.13 ± 45.94	3645.14 ± 22.25
		n-Butanol	760.89 ± 36.20	813.69 ± 1.48	4072.26 ± 110.54	12,399.21 ± 196.75[a]
		Aqueous	705.99 ± 5.42	343.41± 9.03	1415.36 ± 135.04	1227.18 ± 26,.52
T. avicennioides	leaves	Crude	744.29 ± 1.77	704.71 ± 0	5342.85 ± 36.14	4237.23 ± 75.68
		n-Hexane	795.80 ± 7.81	742.17 ± 7.78	1354.51 ± 36.82	1502.13 ± 36,82
		Dichloromethane	247.08 ± 17.57	125.10 ± 6.76	549.91 ± 20.06	373.37 ± 12.29
		Ethyl acetate	832.37 ± 10.84	803.46 ± 1.48	4937.46 ± 216.86	6798.69 ± 184.30
		n-Butanol	866.87 ± 9.03	808.57 ± 1.48	3936.68 ± 168.82	5895.80 ± 61.44
		Aqueous	716.21 ± 1.81	740.30 ± 9.94	3464.4 ± 85.97	3253.17 ± 36.14
	Stem barks	Crude	760.02 ± 1.48	720.90 ± 1.48	4228.53 ± 61.40	4549.83 ± 196.47
		n-Hexane	854.09 ± 45.17	756.62 ± 2.95	2361.75 ± 75.71	3698.92 ± 0
		Dichloromethane	138.73 ± 16.23	788.98 ± 2.56	677.27 ± 17.35	4931.86 ± 0
		Ethyl acetate	816.20 ± 18.10	813.76 ± 1.55	4642.44 ± 70.20	6980.98 ± 49.39
		n-Butanol	858.77 ± 21.28	774.50 ± 1.48	4046.22 ± 24.57	3959.40 ± 147.36
		Aqueous	728.56 ± 1.48	718.34 ± 12.61	7528.11± 134.92[a]	4697.44 ± 135.08
	Roots	Crude	726.43 ± 1.81	680.44 ± 5.42	5357.33 ± 85.97	4011.50 ± 196.47
		n-Hexane	215.76 ± 16.26	835.78 ± 2.95[a]	740.92 ± 36.14	2749.57 ± 26.54
		Dichloromethane	219.59 ± 10.84	810.28 ± 1.48	932.44 ± 43.81	2795.88 ± 24.53
		Ethyl acetate	920.06 ± 41.28[a]	760.02 ± 1.48	3345.79 ± 95.64	5435.46 ± 49.11
		n-Butanol	837.50 ± 46.42	795.80 ± 1.48	5441.25 ± 50.13	6025.84 ± 24.49
		Aqueous	731.12 ± 1.48	754.08 ± 11.50	5166.30 ± 86.97	3658.38 ± 192.08

Values with superscript letters a mean greatest activity.

3.3. Antimicrobial Activity

3.3.1. Inhibition Zone Diameters of Crude Extracts

The effectiveness of crude aqueous and aqueous acetone extracts of *E. hirta* and *T. avicennioides* has been evaluated against 32 causative pathogens of urinary tract infections. It was found an insignificant antimicrobial activity with *E. hirta* extracts (Table 3). The extracts of *T. avicennioides* were effective against 16 strains including 13 bacteria (8 Gram-negative and 5 Gram-positive) and 3 yeasts. The diameters of inhibition zones varied between 7 mm and 16 mm according to the type of plant, organs, extract and microbial strains tested. Aqueous extracts of different parts of *T. avicennioides* and aqueous acetone extracts of leaves and roots of the same species were demonstrated the best results (diameters ≥ 15) against methicillin-resistant *Staphylococcus haemolyticus* and *Candida albicans* strains. Of all these extracts, the aqueous extract of *T. avicennioides* leaves was identified as the most effective.

3.3.2. Efficiency of Fractions of Aqueous Extract *of T. avicennioides* Leaves

The fractions at 10 mg/ml of leaves extract of *T. avicennioides* exhibited variable degrees of antimicrobial activity against certain strains (Table 4). The aqueous fraction of leave was presented the best diameters of inhibition (from 13 mm to 16 mm) against *S. aureus* ATCC 29213, *S. aureus* and the two methicillin-resistant *S. haemolyticus strains*.

3.3.3. Antibacterial Parameters

The values of MIC and MBC/MFC have been determined for the strains which were most susceptible to the aqueous fractions of *T. avicennioides* leaves. The

Table 3. Antimicrobial activity of crude aqueous and hydro-acetonic extracts at 50 mg/ml.

Plants	Organs	Extracts	Zone of inhibition of microbial growth (mm)																
			Gram-negative bacteria								Gram-positive bacteria						Yeast		
			A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
E. hirta	Whole plante	AqAc	/	/	/	/	/	/	10	/	/	/	/	/	8	/	/	/	/
		Aq	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
	Leaves	AqAc	/	/	/	/	11	11	11	10	/	/	11	15	16	7	13	16	10
		Aq	/	/	/	/	11	11	11	10	/	/	14	16	16	/	15	16	9
T. avicennioides	Stem barks	AqAc	/	/	/	/	9	10	9	/	/	/	10	10	14	7	/	/	9
		Aq	10	10	9	/	9	11	11	8	10	12	12	15	14	8	/	/	9
	Roots	AqAc	/	/	/	/	12	12	11	9	11	/	13	15	14	7	/	/	10
		Aq	9	/	/	/	11	12	10	7	10	/	11	15	14	/	/	/	9

AqAc = Aqueous acetone; Aq = Aqueous; A = *k. pneumoniae* strain1; B = *k. pneumoniae* strain2 C = *E. coli* strin3; D = *Enterobacter aeroginosa*; E = *Acinetobacter baumannii* strain1 F = *Acinetobacter baumannii* strain2; G = *Klebsiella oxytoca*; H = *Pseudomonas aeruginosa*; I = *Proteus mirabilis*; J = *S.aureus* ATCC 29213; K = *S. aureus;* L = methicillin-resistant *Staphylococcus haemolyticus* strain1; M = methicillin-resistant *Staphylococcus haemolyticus* strain2; N = *Streptococcus agalactiae*; O = *Candida albicans* strain1; P = *Candida albicans* strain2; Q = *Candida albicans* strain 3.

aqueous fraction of leaves has shown a bactericidal effect with a concentration of MIC (5 mg/ml) and of MBC (10 mg/ml) against strains recorded in (Table 5).

3.3.4. Synergy Assay

Table 6 indicate the interaction between 9 antibiotic discs and aqueous fraction of *T. avicennioides leaves* against methicillin-resistant *S. haemolyticus* 1 and 2; *S. aureus* and *S. aureus* ATCC 29213. The extract has exhibited synergistic effects

Table 4. Antimicrobial activity of fractions from leaves aqueous crude extracts.

Fractions	Zone of inhibition of microbial growth (mm)																
	Gram-negative bacteria								Gram-positive bacteria					Yeast			
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
n-Hexane	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Dichloromethane	/	/	/	/	/	/	/	/	/	/	8	/	/	/	/	/	/
Ethyl acetate	/	/	/	/	10	/	/	/	/	/	17	/	/	/	/	/	/
n-Butanol	/	/	/	/	10	/	/	/	/	/	15	/	14	/	/	/	/
Aqueous	/	/	/	/	/	/	/	/	/	16	16	14	13	/	10	11	10

Table 5. Antibacterial parameters of aqueous fraction of *T. avicennioides* leaves.

Strains	MIC	MBC	MBC/MIC	Effects
Staphilococcus haemolyticus 1	5	10	2	Bactericidal
Staphilococcus haemolyticus 2	5	10	2	Bactericidal
Staphilococcus aureus	5	10	2	Bactericidal
*Staphilococcus aureus*ATCC 29213	5	10	2	Bactericidal

Table 6. Mean zone of inhibition (mm) of different antibiotics (without and with extract) against bacteria.

Antibiotic	*S. haemoliticus* 1		*S. haemoliticus* 2		*S. aureus*		*S. aureus* ATCC 29213	
	A	B	A	B	A	B	A	B
Trimethoprim (25 µg)	26	28	14	19	25	23	31	33
Fosfomycin (200 µg)	19	19	14	13	34	32	18	22
Cefoxitin (30 µg)	13	18	13	20	28	26	23	28
Penicillin G (10 µg)	R	10	R	11	11	11	20	14
Tetracycline (30 µg)	R	11	R	10	R	11	27	29
Clindamycin (10 µg)	34	33	12	13	31	30	31	33
Erythromycin (15 µg)	30	31	31	31	28	20	25	29
Gentamycin (10 µg)	30	29	17	11	26	21	23	23
Chloramphenicol (30 µg)	27	28	28	27	27	23	28	28

A = Inhibition zone of disc without extract; B = Inhibition zone of disc associated to extract; zones of inhibition highlighted mean a synergetic effect.

with some antibiotic. The best improvement of the diameter was obtained with Penicillin G (10 µg) and Tetracycline (30 µg) followed by Cefoxitin (30 µg).

4. Discussion

The quantitative analysis of total phenolic, flavonoids and tannins contents of *E. hirta* and *T. avicennioides* indicated variable contents with the vegetable species, parts and solvent used. This result could be linked to extraction solvent polarity, environmental conditions and genetic factors. The greatest polyphenol content was found in n-Butanol fraction from *E. hirta*. Ethyl acetate fraction of *E. hirta* yielded the best amount in total flavonoids. Aqueous acetone extract from *T. avicennioides* stem barks was most riche in total tannins. Many beneficial effects derived from phenolics have been attributed to their antioxidant activity [20]. Several analytical methods have been carry out to explore the antioxidant properties of plant extracts [21].

In this study free radicals scavenging (DPPH) and ferric reducing antioxidant power (FRAP) methods have been used to assess the antioxidant activities of crude extracts and fractions of *E. hirta* and *T. avicennioides*. All the extracts demonstrated antioxidant activities. The best antioxidant capacities were obtained with Ethyl Acetate fraction from *T. avicennioides* roots in DPPH assay; and n-Butanol fraction from *E. hirta* in FRAP testing. These activities within the extracts could be due to high correlations between polyphenols contents and antioxidant capacity [22] [23]. Thus, the direction and the magnitude of the correlation were calculated in order to estimate the correlation between the phenolics contents and free radicals scavenging activity or ferric reducing power.

In this work, a high correlation was demonstrated between the total polyphenol content and DPPH scavenging activity ($p = 3.089e^{-12}$, $r^2 = 0.7281619$) or ferric reducing power ($p < 2.2e^{-16}$, $r^2 = 0.9508803$). There was a moderate correlation of total flavonoids levels with antioxidant capacity in DDPH method ($p = 7.405^{e-07}$, $r^2 = 0.4257956$) and FRAP assay ($p = 7.339e^{-10}$, $r^2 = 0.5629441$). A weak correlation of total tannins was shown with Both DPPH ($p = 0.1035$, $r^2 = 0.1468837$) and FRAP ($p = 0.02893$, $r^2 = 0.2163953$) antioxidant tests. This variation could be explaining by some phenolic intrinsic factors such as: structure-activity relationships, polymerization grade and possible synergy or antagonism among different classes of compounds [24]. Antioxidant properties of phenolic compounds could be an advantage for further anti-bacterial compounds investigation.

Regarding antibacterial activity, crude extracts (at 50 mg/ml) and a fraction (at 10 mg/ml) from *E. hirta* and *T. avicennioides* have been evaluated against 32 causative pathogens of urinary tract infections. The extracts of *T. avicennioides* were effective against 16 strains. This difference in results between the two species could be linked to the environmental conditions of both plants and the polarity of the extraction solvents. Of all extracts from *T. avicennioides*, aqueous fraction of *T. avicennioides* leaves allowed the best results (diameter from 13 mm

to 16 mm) against *S. aureus* ATCC 29213, *S. aureus* and 2 methicillin-resistant *Staphylococcus haemolyticus* strains. This result could be explained by a concentration of the active molecules in this fraction.

A further antibacterial parameters study revealed the bactericidal effect (CMB/CMI < 4) of this fraction. Other studies realized in Nigeria have also proven anti-methicillin resistant activity of certain extracts from *T. avicennioides* [25] [26]. These results prove anti-methicillin resistant property of *T. avicennioides* and its potency to be efficient in inhibiting the UTI causing pathogens. The antimicrobial activity of this plant could be attributed to the presence of tannins, flavonoids and phenols.

Aqueous fraction of *T. avicennioides* leaves was then used to screen synergistic action with antibiotic discs. It was found in this study the increasing of the size of some inhibition zones, involving the synergic effect. Several studies have reported the potentiation of antibiotic effect with phytochemicals [27] [28]. This could be due to the mechanism of action of drugs against organisms used and proper selection of natural compounds [29]. There is a need for more studies concerning the molecular basis of synergistic interactions for the development of novel therapies against methicillin-resistant and multi-resistant strains in general.

5. Conclusions

The present study revealed that *E. hirta* and *T. avicennioides* exhibited variable degrees of antioxidant potentials in DPPH and FRAP assays.

Great to weak correlation were demonstrated between this activity and total phenolics, flavonoids and tannins contents. The antimicrobial activity of these plants could be attributed to the presence of phenolics.

The extracts of *T. avicennioides* were effective against 16 causative pathogens of urinary tract infections; and its leaves allowed bactericidal effect against *S. aureus* ATCC 29213, *S. aureus* and 2 methicillin-resistant *Staphylococcus haemolyticus* strains. Further studies must be done in order to identify the bioactive molecules. *T. avicennioides* leaves could be recommended as an herbal drug formulation for treating UTI.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ditkoff, E.L., Theofanides, M., Aisen, C.M., Kowalik, C.G., Cohn, J.A., Sui, W., *et al.* (2018) Assessment of Practices in Screening and Treating Women with Bacteriuria.

Canadian Journal of Urology, **25**, 9486-9496.

[2] Al-Gasha'a, F.A.S., Al-Baker, S.M., Obiad, J.M. and Alrobiai, F.A. (2020) Prevalence of Urinary Tract Infections and Associated Risk Factors among Patients Attending Medical City Hospital in Baghdad City, Iraq. *American Journal of Infectious Diseases*, **16**, 77-84. https://doi.org/10.3844/ajidsp.2020.77.84

[3] Mickymaray, S. and Al-Aboody, M.S. (2019) *In Vitro* Antioxidant and Bactericidal Efficacy of 15 Common Spices: Novel Therapeutics for Urinary Tract Infections? *Medicina*, **55**, Article 289. https://doi.org/10.3390/medicina55060289

[4] WHO (2014) Antimicrobial Resistance: Global Report on Surveillance. World Health Organization. https://apps.who.int/iris/handle/10665/112642

[5] Mandal, S.M., Dias, R.O. and Franco, O.L. (2017) Phenolic Compounds in Antimicrobial Therapy. *Journal of Medicinal Food*, **20**, 1031-1038.

[6] Rahman, M., Rahaman, S., Islam, R., Rahman, F., Mithi, F.M., Alqahtani, T., *et al.* (2022) Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. *Molecules*, **27**, Article 233. https://doi.org/10.3390/molecules27010233

[7] Wang, H.C. and Brumaghim, J.L. (2011) Polyphenol Compounds as Antioxidants for Disease Prevention: Reactive Oxygen Species Scavenging, Enzyme Regulation, and Metal Chelation Mechanisms in *E. coli* and Human Cells. In: Andreescu, S. and Hepel, M., Eds., *Oxidative Stress: Diagnostics, Prevention, and Therapy*, American Chemical Society, New York, 99-175. https://doi.org/10.1021/bk-2011-1083.ch005

[8] Omwenga, E.O., Hensel, A., Shitandi, A. and Goycoolea, F.M. (2015) Ethnobotanical Survey of Traditionally Used Medicinal Plants for Infections of Skin, Gastro Intestinal Tract, Urinary Tract and the Oral Cavity in Borabusubcounty, Nyamira County, Kenya. *Journal of Ethnopharmacology*, **176**, 508-514. https://doi.org/10.1016/j.jep.2015.11.032

[9] Mahalik, G., Sahoo, S. and Satapathy, K.B. (2015) Ethnobotanical Survey of Plants Used in Treatment of Urinary Disorders in Dhenkanal District of Odisha, India. *IOSR-JESTFT*, **9**, 58-63.

[10] Jaradat, N.A., Zaid, A.N., Al-Ramahi, R., Alqub, M.A., Hussein, F., Hamdan, Z., *et al.* (2017) Ethnopharmacological Survey of Medicinal Plants Practiced by Traditional Healers and Herbalists for Treatment of Some Urological Diseases in the West Bank/Palestine. *BMC Complementary and Alternative Medicine*, **17**, Article No. 255. https://doi.org/10.1186/s12906-017-1758-4

[11] Nacoulma, O.G. (1996) Plantes médicinales et Pratiques médicinales Traditionnelles: Cas du plateau central. Ph.D. Thesis, Universite de Ouagadougou, Burkina Faso.

[12] Meda, N.T.R., Lamien-Meda, A., Kiendrebeogo, M., Lamien, C.E., Coulibaly, A.Y., Millogo, R., *et al.* (2010) *In Vitro* Antioxydant, Xanthine Oxidase and et Acetylcholinesterase Inhibitory Activities of *Balanites aegyptiaca* (L.) Del. (Balanitaceae). *Pakistan Journal of Biological Sciences*, **13**, 362-368. https://doi.org/10.3923/pjbs.2010.362.368

[13] Agbangnan, P.D., Tachon, C., Bonin, H., Chrostowska, A., Fouquet, E. and Sohounhloue, C.K.D. (2012) Phytochemical Study of a Tinctorial Plant of Benin Traditional Pharmacopoeia: The Red Sorghum (*Sorghum caudatum*) of Benin. *Scientific Study & Research*, **13**, 121-135.

[14] Murray, P., Baron, E., Pfaller, M., Tenover, F. and Yolke, R. (1995) Manual of Clinical Microbiology. 8th Edition, ASM, Washington DC.

[15] Ponce, A.G., Fritz, R., del Valle, C. and Rouras, I. (2003) Antimicrobial Activity of Essential Oils on the Native Microflora of Organic Swiss Chard. *LWT-Food Science*

and Technology, **36**, 679-684. https://doi.org/10.1016/S0023-6438(03)00088-4

[16] Balouiri, M., Sadiki, M. and Ibnsouda, S.K. (2016) Methods for *in Vitro* Evaluating Antimicrobial Activity: A review. *Journal of Pharmaceutical Analysis*, **6**, 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[17] Sanogo, Y., Guessennd, N.K., Tra Bi, H.F., Kouadio, N.J., Konan, F.K., Bamba, M., *et al.* (2016) Evaluation *in Vitro* de l'activité des écorces de tige de *Anogeissus leiocarpus* (DC) Guill. et Perr. (Combretaceae) sur des bactéries responsables de maladies courantes en Afrique et criblage phytochimique. *International Journal of Biological and Chemical Sciences*, **10**, 1139-1152. https://doi.org/10.4314/ijbcs.v10i3.19

[18] Eberlin, T. (1994) Les antibiotiques Classification, mode d'action, utilisation thérapeutique. Nathan, Paris.

[19] Chaudhary, R. (1996) Herbal Drugs Industry: A Practical Approach to Industrial Pharmacognosy. 1th Edition, Eastern Publishers.

[20] Kumar, S., Abedin, M., Singh, A.K. and Das, S. (2020) Role of Phenolic Compounds in Plant-Defensive Mechanisms. In: Lone, R., Shuab, R. and Kamili, A., Eds., *Plant Phenolics in Sustainable Agriculture*, Springer, Singapore, 517-532. https://doi.org/10.1007/978-981-15-4890-1_22

[21] Magalhães, L.M., Segundo, M.A., Reis, S. and Lima, J.L. (2008) Methodological Aspects about *in Vitro* Evaluation of Antioxidant Properties. *Analytica Chimica Acta*, **613**, 1-19. https://doi.org/10.1016/j.aca.2008.02.047

[22] Anesini, C., Graciela, F.E. and Filip, R. (2008) Total Polyphenol Content and Antioxidant Capacity of Commercially Available Tea (*Camellia sinensis*) in Argentina. *Journal of Agricultural and Food Chemistry*, **56**, 9225-9229. https://doi.org/10.1021/jf8022782

[23] Madrigal-Carballo, S., Rodriguez, G., Krueger, C.G., Dreher, M. and Reed, J.D. (2009) Pomegranate (*Punica granatum*) Supplements: Authenticity, Antioxidant and Polyphenol Composition. *Journal of Functional Foods*, **1**, 324-329. https://doi.org/10.1016/j.jff.2009.02.005

[24] Majo, D.D., La Guardia, M., Giammanco, S., La Neve, L. and Giammanco, M. (2008) The Antioxidant Capacity of Red Wine in Relationship with Its Polyphenolic Constituents. *Food Chemistry*, **111**, 45-49. https://doi.org/10.1016/j.foodchem.2008.03.037

[25] Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. (2005) Screening of Crude Extracts of Six Medicinal Plants Used in South-West Nigerian Unorthodox Medicine for Anti-Methicillin Resistant *Staphylococcus aureus* Activity. *BMC Complementary and Alternative Medicine*, **5**, Article No. 6. https://doi.org/10.1186/1472-6882-5-6

[26] Ugochukwu, A.E., John, D.E., Amin, A.F., Chidi, E., Nwobodo, N.N., Ogbonna, O.R., *et al.* (2018) Bacteriostatic and Bactericidal Effects of Ethyl Acetate Root Bark Extract of *Terminalia avicennioides* on Methicillin-Resistant *Staphylococcus aureus*. *African Journal of Biochemistry Research*, **12**, 45-54. https://doi.org/10.5897/AJBR2017.0981

[27] Khameneh, B., Iranshahy, M., Soheili, V., Sedigheh, B. and Bazzaz, F. (2019) Review on Plant Antimicrobials: A Mechanistic Viewpoint. *Antimicrobial Resistance and Infection Control*, **8**, Article No. 118. https://doi.org/10.1186/s13756-019-0559-6

[28] Oseni, A., Olusola-Makinde, O. and Oladunmoye, M. (2021) Structural Elucidation, Mechanism of Action, Antibacterial Proficiency and Synergistic Forte of Purified *Euphorbia hirta* Whole Plant Extract against Multi-Drug Resistant Otitis Media In-

fection. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, **20**, 575-597. https://doi.org/10.37360/blacpma.21.20.6.42

[29] Chanda, S. and Rakholiya, K.D. (2011) Combination Therapy: Synergism between Natural Plant Extracts and Antibiotics against Infectious Diseases. *Formatex*, **5**, 520-529.