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Synergistic Antibacterial Screening of *Cymbopogon citratus* and *Azadirachta indica*: Phytochemical Profiling and Antioxidant and Hemolytic Activities

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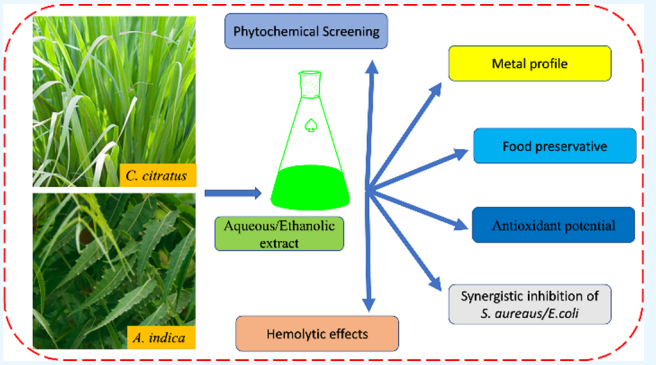
ABSTRACT: Current studies were performed to investigate the phytochemistry, synergistic antibacterial, antioxidant, and hemolytic activities of ethanolic and aqueous extracts of *Azadirachta indica* (EA and WA) and *Cymbopogon citratus* (EC and WC) leaves. Fourier transform infrared data verified the existence of alcoholic, carboxylic, aldehydic, phenyl, and bromo moieties in plant leaves. The ethanolic extracts (EA and EC) were significantly richer in phenolics and flavonoids as compared to the aqueous extracts (WA and WC). The ethanolic extract of *C. citratus* (EC) contained higher concentrations of caffeic acid (1.432 mg/g), synapic acid (6.743 mg/g), and benzoic acid (7.431 mg/g) as compared to all other extracts, whereas chlorogenic acid (0.311 mg/g) was present only in the aqueous extract of *A. indica* (WA). Food preservative properties of *C. citratus* can be due to the presence of benzoic acid (7.431 mg/g). —Gas chromatography−mass spectrometry analysis demonstrated the presence of 36 and 23 compounds in *A. indica* and *C. citratus* leaves, respectively. Inductively coupled plasma analysis was used to determine the concentration of 26 metals (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Sn, Sr, V, Zn, Zr, Ti); the metal concentrations were higher in aqueous extracts as compared to the ethanolic extracts. The extracts were generally richer in calcium (3000−7858 ppm), potassium (13662−53,750 ppm), and sodium (3181−8445 ppm) and hence can be used in food supplements as a source of these metals. Antioxidant potential (DDPH method) of *C. citratus* ethanolic extract was the highest (74.50 ± 0.66%), whereas it was the lowest (32.22 ± 0.28%) for the aqueous extract of *A. indica*. Synergistic inhibition of bacteria (*Staphylococcus aureus* and *Escherichia coli*) was observed when the aqueous extracts of both the plants were mixed together in certain ratios (v/v). The highest antibacterial potential was exhibited by the pure extract of *C. citratus*, which was even higher than that of the standard drug (ciprofloxacin). The plant extracts and their mixtures were more active against *S. aureus* as compared to *E. coli*. No toxic hemolytic effects were observed for the investigated extracts indicating their safe medicinal uses for human beings.



INTRODUCTION

Plants are largely investigated especially due to their valuable nutrients,[1,2] useful phyto-chemicals,[3,4] minimal side effects,[1,2,5] more satisfaction to the users,[6] and significant medicinal properties[5,7] including antidiabetic, thrombolytic, antimicrobial, antioxidant, etc.[8] In developing countries, approximately 80% of the population is dependent on herbal medicines for their primary health care needs.[9,10]

Azadirachta indica (Neem or Arista native to India) is a medicinal plant that is mainly cultivated in agricultural lands and homes of the tropical and subtropical regions.[11,12] Its leaves are rich in vitamin C, protein, carbohydrates, minerals, glutamic acid, carotene, tyrosine, aspartic acid, praline, glutamine, and cysteine.[13] The active ingredients in neem include nimbin, nimbidin, nimbidol, sodium nimbinate gedunin, salannin, quercetin, and nimbolinin.[8] Its leaves display antioxidant,[14] anti-inflammatory,[15] antibacterial, antiseptic, healing, and anthelmintic potential.[16] They are also effective against many chronic and microbial diseases, ulcer, inflammation, constipation, bloating and cramping, syphilis, bleeding nose, intestinal worms, belching, nausea, anorexia, gastric difficulties, lack of appetite, birth control, cardiovascular disorders, hypoglycemia, oral infections, liver problems, skin ulcers,[17] and skin diseases like psoriasis and eczema.[18] Neem

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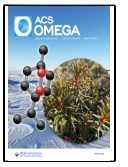

Table 1. FTIR (cm^{-1}) Data of Aqueous and Ethanol Extracts of *A. indica* and *C. citratus*

A. indica (A)			*C. citratus* (C)		
ethanolic extract (EA)	aq. extract (WA)	functional group	ethanolic extract (EC)	aq. extract (WC)	functional group
614	625	C−Br	626	622	C−Br
1026	1044	C−N	877	859	C−H Ar
1364	1394	C−H−CH_3	1419	1398	C−H Aldehyde
1635	1575	−$COO_{stretch}$	2973	2915	C−H alkanes
3261 (broad)	3234 (broad)	O−H alcoholic or carboxylic	3332 (broad)	3273 (broad)	O−H alcoholic or carboxylic

oil is used for improvement of liver function, blood detoxification, and regulation of the blood sugar level.[18] However, neem is a less effective medication in patients having organ rejection.[19] The plant also finds uses in soaps, shampoo, toothpaste, and conditioners.[16] Neem extract capsules are used as a hair and skin care supplement.[20] Its flowers are sweet and used to cook many dishes in south India.[17] The dried neem leaves are placed in closets, cupboards, grain bins, books, beds,[21] and rice tins[22] because they prevent the spoilage of grains/items by acting as nontoxic pest repellents, sterilants, and antifungal and antiparasitic agents.[6] Neem acts as an egg-laying deterrent and also suppresses the hatching of pest insects from their eggs. It acts as an antifeedant so the crop insects are starved and ultimately killed.[18] The use of neem is effective to control foliar diseases of crops and increases the grain yield as an agricultural fertilizer.[23]

Cymbopogon citratus, a plant native to Sri Lanka and southern India, is found/cultivated indoors or outdoors in South and Central America, in Savannah, tropical and subtropical regions.[24,25] *C. citratus* contains alcohols, ketones, esters, aldehydes, saponins, terpenoids, terpenes, neryl, nerol, myrcene, geraniol, citral, gernyl acetate, and many flavonoids.[26] The composition of *C. citratus* essential oils varies from region to region.[27] Its leaves have unique lemon scent due to the presence of citral.[25,27] The plant contains carbohydrates, crude proteins, crude ash, fat, and several polyphenolic substances, e.g., luteolin, apiginin, flavonoids, apigerol, caffeic acid, catechol, and elimicin.[28] Its phenolic contents, essential oils, and aqueous/other extracts are commercially used in pharmaceuticals, cosmetics, and perfumery.[24] Lemongrass has anti-inflammatory and anticancer properties due to the presence of citral and geranial components.[29] Its roots can be used as a mouth cleaning agent and also to reduce fever intensity. *C. citratus* is used in frozen desserts, baked food items, gelatin, meat, pudding, sauces, and wine.[30] Its oil may be used to treat drug-resistant bacterial infections, insomnia, respiratory disorders, and bad body odor.[25] Due to its antibacterial and cleansing properties, it is used in the manufacture of deodorants and sanitizers for smelly feet. It can be applied as a mosquito repellent due to its antiprotozoan properties and also used in shampoos to get rid of dandruff and lice.[31] *C. citratus* helps to preserve the food quality and freshness by reducing lipid oxidation and rancidity.[32]

It is well known that the phytochemical composition of the same plant species may vary from region to region.[27] Numerous studies were reported earlier regarding the phytochemistry, antioxidant, and antimicrobial potential of *A. indica*[33−35] and *C. citratus*[28,36,37] but there are still no reports on the synergistic antibacterial potential of these plants. Since plants produce different phytochemicals, the extract combinations of various plants may work together more efficiently to fight against different pathogens.[38] Keeping in view the common occurrence and broad-spectrum uses of *A. indica* and *C. citratus*, current studies were performed to investigate the synergistic antibacterial potential of the aqueous and ethanolic extracts of their leaves. The plant extracts were also subjected to phytochemical screening, antioxidant, and hemolytic activity studies.

RESULTS AND DISCUSSION

Plant extracts of *A. indica* and *C. citratus* were prepared and analyzed for phytochemical ingredients and metal analysis. The ethanol and water solvents were used for plant extraction because both these solvents have different polarity and also acceptable for human consumption.[39,40] They can be safely introduced into the useful products without any risk of unacceptable level of hazardous solvent residues or disturbance of nutritional quality of food products.[40,41] The prepared extracts were also investigated for their antioxidant effects, synergistic biofilm inhibition of bacteria, and hemolytic activities.

Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra of aqueous and ethanolic extracts of both the plants (*A. indica* and *C. citratus)* were recorded in the range of 4000−600 cm^{-1}. The spectral data are displayed in Table 1 while the spectra are shown in Figures S1−S4 (Supplementary material).

The aqueous and ethanolic extract of *A. indica* showed the presence of C−Br, C−N, C−H of the −CH_3 group, −COO stretching of the carboxylate group, and O−H vibrations of alcohol. FTIR spectroscopy displayed a broad band at 3261 and 3234 cm^{-1} in ethanolic (EA) and aqueous extracts (WA), respectively of *A. indica*; these bands were assigned to the O−H stretching vibrations of alcohols/carboxylic acids. The strong absorption peaks at 1635 cm^{-1} in EA and 1575 cm^{-1} in WA can be assigned to the C═O asymmetric stretching vibrations of the carboxylate groups (COO−). The peaks at 1364 (EA) and 1394 cm^{-1} (WA) were due to the deforming vibrations of the aldehydic C−H bonds. The vibrational bands at 1026 (EA) and 1044 cm^{-1} (WA) in ethanolic and aqueous extracts, respectively, indicate the presence of C−N bonds. The bands at 614 cm^{-1} (ethanolic extract) and 625 cm^{-1} (aqueous extract) indicate the presence of halo compounds in leaf extracts of *A. indica*.

Peaks at 3332, 2973, 1419, 877, and 626 cm^{-1} were displayed in the ethanolic extract of *C. citratus* and at 3273, 2915, 1398, 859, and 622 cm^{-1} in the aqueous extract of the same plant (Table 1). FTIR spectra have shown a broad band at 3332 (EC) and 3273 cm^{-1} (WC) which can be assigned to the stretching vibration of hydrogen bonded O−H groups of alcohols/carboxylic acids. Strong-intensity peaks in the EC and WC at 2973 and 2915 cm^{-1}, respectively, were recognized as C−H bands of alkanes. The absorption peaks at 1419 cm^{-1} (EC) and 1398 cm^{-1} (WC) represent the aldehydic C−H (stretching) bonds. The bands at 877 cm^{-1} (EC) and 701 cm^{-1} (WC) were due to the aromatic C−H vibrations. The

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Table 2. TFC and TPC in Plant Extracts[a]

sample	TFC as mg CE/100 g of plant extract (where CE = Catechin equivalent)	TPC as mg GAE/100 g of plant extract (where GE = Gallic acid equivalent)
ethanolic extract of *C. citratus* (EC)	90.56 ± 0.81	131.98 ± 1.12
aqueous extract of *C. citratus* (WC)	22.69 ± 0.17	38.95 ± 0.25
ethanolic extract of *A. indica* (EA)	100.56 ± 1.13	65.70 ± 0.56
aqueous extract of *A. indica* (WA)	23.76 ± 0.19	14.53 ± 0.12

[a]The values were the average of triplicate samples (n = 3) ± S.D., two-way analysis of variance (ANOVA) shows ($P \leq 0.05$), so results are significant.

bromo groups displayed the peaks at 626 cm^{-1} (ethanolic extract) and 622 cm^{-1} (aqueous extract) (Table 1).

Quantitative Determination of the Total Flavonoid Content (TFC) and Total Phenolic Content (TPC). TFCs and TPCs were calculated in terms of milligrams (mg) of catechin equivalents (CE) and gallic acid equivalents (GAE), respectively, per 100 grams of the plant extract. The obtained data are given in Table 2.

C. citratus and *A. indica* were found to possess variable quantities of flavonoids and phenolics depending upon the nature of plant species and the solvent sources used for their extraction. The ethanolic extracts were richer in the TFC as well as in TPC as compared to their aqueous counterparts in both the plants (Table 2). The ethanolic extract (EC) of *C. citratus* leaves was found to contain higher concentration of TFC (90.56 mg CE/100 g of extract) as compared to that (22.69 mg CE/100 g of extract) of its aqueous counterpart (WC). Similarly, the TFC was present in higher concentration (100.56 mg CE/100 g of extract) in the ethanolic extract (EA) of *A. indica* as compared to those (23.76 mg CE/100 g of extract) present in its aqueous extract (WA). The ethanolic extract (EC) of *C. citratus* leaves was also richer (131.98 mg GAE/100 m of extract) in TPC as compared to its aqueous extract (WC) (38.95 mg GAE/100 g of extract). Similarly, *A. indica* ethanolic extract (EA) also possessed a higher concentration of TPC (65.70 mg GAE/100 g of extract) as compared to that (14.53 mg GAE/100 g of extract) of its aqueous extract (WA) (Table 2).

The phenolic compounds play an important role in human diet as antioxidants and are present abundantly in plants (vegetables and fruits), beverages, and byproducts from agriculture and food.[42] Flavonoids are natural polyphenolic compounds that produce the color and taste in various parts of herbs, vegetables, plants, and fruits. They find important medicinal value due to their antioxidation, anticancer, anti-inflammation, antiobesity, antidiabetes, neuroprotective, anti-mutagenic properties, etc.[43] The reported literature also suggests that *C. citratus* is rich in TFC and TPC.[44,45] Their presence in *A. indica* has also been suggested in earlier studies.[46,47]

Quantitative Analysis of Phenolics and Flavonoids by HPLC. For HPLC analysis, various parameters were studied using phenolic and flavonoids standards. The limit of detection (LOD) and the limit of quantification (LOQ) of the developed method were calculated from the equation of line obtained from the calibration curve (Table 3A). The HPLC data are shown in Table 3B, whereas the corresponding spectra are displayed in Figures S5−S8 (for phenolics) and S9−S12 (for flavonoids) of the Supplementary Material.

Table 3A. HPLC Parameters for Standards

standards	LOD (ppm)	LOQ (ppm)	R^2
gallic acid	0.27	0.81	0.9981
chlorogenic acid	0.19	0.51	0.9984
caffeic acid	0.17	0.60	0.9972
synapic acid	0.24	0.72	0.9965
benzoic acid	0.22	0.77	0.9975
myricetin	0.21	0.67	0.9988
quercetin	0.17	0.53	0.9912
kaempferol	0.10	0.41	0.9871

Different concentrations of phenolics were observed in the investigated leaf extracts of *A. indica* and *C. citratus* (Table 3B). The results of HPLC analysis clearly demonstrate the presence of gallic acid, caffeic acid, and synapic acid in all the leaf extracts except the ethanolic extract of *C. citratus* (Table 3B). The chlorogenic acid (0.311 mg/g) was present only in the aqueous extract of *A. indica*. The ethanolic extract of *C. citratus* contained higher concentrations of caffeic acid (1.432 mg/g), synapic acid (6.743 mg/g), and benzoic acid (7.431 mg/g) as compared to all other extracts. Since the ethanolic extract of *C. citratus* was richer in benzoic acid (7.431 mg/g), so the said extract can be applied as a natural food preservative.[48] Food preservative properties can also be displayed by the aqueous extract of *A. indica* having 0.197 mg/g concentration of benzoic acid (Table 3A).

The presence of different flavonoids (myricetin, quercetin, and kaempferol, Table 3B) was also determined in investigated leaf extracts. The ethanolic extract of *C. citratus* was rich in myricetin (7.147 mg/g) with some quantities of quercetin (0.246 mg/g) and kaempferol (0.180 mg/g). The ethanolic extract of *A. indica* leaves contained only myricetin (12.438 mg/g). No flavonoid content was detected in any aqueous extract whether it belonged to *A. indica* or *C. citratus* (Table 3B).

Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. GC−MS analysis of aqueous/ethanolic extracts of *A. indica* and *C. citratus* has shown the presence of numerous volatile compounds which are displayed in Table 4 while the corresponding spectra are shown in Figures S13−S16.

The GC−MS results revealed the presence of many bioactive compounds which occur with their specific respective retention times and peak areas. The important biologically active compounds include thymine,[49] 4-piperidinone,[50] phytol,[51] vitamin E,[52] campesterol, stigmasterol, sitosterol,[53] cannabidiol,[52] and lanosterol[54] in ethanolic extracts of *A. indica* while mequinol,[55] glutarimide (an antibiotic),[56] adrenaline (a physiological metabolic regulatory hormone in humans),[57] guanine,[58] etc. were present in its aqueous extract. The

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Table 3B. Phytochemical Concentration (mg/g) in *A. indica* and *C. citratus* Leaf Extracts[a]

phytochemical constituent	ethanolic extract of *C. citratus* (EC)	aqueous extract of *C. citratus* (WC)	ethanolic extract of *A. indica* (EA)	aqueous extract of *A. indica* (WA)
		phenolic contents (mg/g)		
gallic acid	0.255 ± 0.002	ND	0.289 ± 0.031	0.197 + 0.001
chlorogenic acid	ND	ND	ND	0.311 + 0.002
caffeic acid	1.432 ± 0.001	ND	0.490 ± 0.043	0.192 + 0.004
synapic acid	6.743 ± 0.051	ND	1.8420.043	0.986 + 0.008
benzoic acid	7.341 ± 0.065	ND	ND	0.197 + 0.002
		flavonoid contents (mg/g)		
myricetin	7.147 + 0.073	ND	12.438 + 0.130	ND
quercetin	0.246 + 0.002	ND	ND	ND
kaempferol	0.180 + 0.12	ND	ND	ND

[a]The values were the average of triplicate samples (n = 3) ± S.D., two-way ANOVA shows ($P \leq 0.05$) at a confidence interval 95%, so results are significant; ND = Not detected.

Table 4. Phytochemicals in the Ethanolic/Aqueous Extract (EA) of *A. indica* and *C. citratus*

ethanolic extract (EA) of *A. indica*		aqueous extract (WA) of *A. indica*		ethanolic extract (EC) of *C. citratus*		aqueous extract (WC) of *C. citratus*	
peak area%	compounds present	peak area%	compounds present	peak area%	compounds present	peak area%	compounds present
0.83	thymine	3.10	propanoic acid, 2-methyl-, butyl ester	0.76	tetradecanol	6.98	cyclopentasiloxane, decamethyl-
0.60	2-methoxy-N-, methyethylamine	3.58	mequinol	0.63	Geraniol	5.63	cyclohexasiloxane, dodecamethyl-
0.77	4-piperidinone, 1,2,5- trimethyl	6.36	1,4-butanediamine	0.80	grandisol	2.82	cis-linaloloxide
1.59	5hydroxymethylfurfural	0.58	diaziridine,1,3,3-trimethyl-	0.73	neric acid	2.80	trans-linalool oxide
3.76	*n*-hexadecanoic acid	2.89	cyclopentasiloxane, decamethyl-	1.45	Allyldimethylsilane	4.84	pentasiloxane, dodecamethyl-
19.99	phytol	0.25	4-oxo-trans-2-octenal	0.73	tetradecanoic acid	2.22	cyclooctasiloxane, hexadecamethyl-
1.03	methyl 2,3-dimethyl-3-nitrosobutanoate	0.44	tetrahydropyran	1.94	neophytadiene	42.45	hexadecanoic acid, methyl ester
2.66	octadecanoic acid	0.48	glutarimide	15.95	n-hexadecanoic acid	23.15	3 methyl stearate
2.36	vitamin E	0.67	adrenaline	1.75	Phytol	1.35	hexasiloxane, tetradecamethyl-
1.07	campesterol	0.25	2 dodecanone	0.52	octadecanoic acid		
2.46	stigmasterol	0.28	guanine	1.37	eicosanoic acid		
7.17	beta sitosterol	0.81	5-hepten-2-one	0.83	1-hexacosene		
0.96	cannabidiol	0.41	1 hexacosanol	1	Cholesterol		
4.25	9,12,15-octadecatrien-1-ol,	0.76	6-dodecene	1.11	Nandrolone		
2.45	bis(2-ethylhexyl) phthalate	0.26	stibine, triphenyl-				
2.36	(1-cyclopropylimidazol-2-yl) methanamine	0.49	hexaphenylditin				
1.77	9,19-cyclolanost-24-en-3-ol, (3 beta)						
1.29	lanosterol						
0.92	9,19-cyclolanost25-en-3-ol, 24-methyl						
1.41	vitamin E						

biologically active geraniol,[59] grandisol (the boll weevil pheromone),[60] neric acid (a pheromone),[61] neophytadiene,[62] *n*-hexadecanoic acid,[63] phytol,[62] octadecanoic acid (anti-inflammatory, antioxidant, anticancer),[64] hexacosene,[65] and nandrolone (anticancer)[66] were observed in the investigated ethanolic extract of *C. citratus* while its aqueous extract contained cyclopentasiloxane (antioxidant),[67] cis-linalool oxide (antifungal and antitermitic),[68] trans-linalool oxide (pyranoid),[69] pentasiloxane,[67] hexadecanoic acid,[63,67] and hexasiloxane (antieczemic, anticoranary, antiantrogenic, and nematicide).[70] Geraniol is the main bioactive component of *C. citratus*,[59] which shows a variety of activities including repellence to sand flies, mosquitoes, cattle ticks,[71] prevention of secondary complications in diabetes and reduction of hyperglycaemia,[72] anti-inflammatory and antioxidant properties,[73] antifungal activity,[74] activity against respiratory pathogens, skin and food-derived strains, potential against prostate/kidney/liver/bowel and skin cancer,[73] and antibacterial properties especially against *B. subtilis* (a pathogen of the bakery industry).[75] Geraniol also finds applications as a fragrance component in cosmetic and household products.[73]

The comparison of data in Table 4 clearly indicates that the ethanolic extract of each plant is richer in phytochemicals as compared to its corresponding aqueous extract. It justifies the higher antioxidant and antibacterial efficacy of ethanolic extracts which has been discussed further in incoming discussion sections of "Antioxidant Activity by DPPH Method" and "Synergistic Antibacterial Potential".

Metal Analysis. *Metal Detection in the Extracts.* Metal analysis of aqueous and ethanolic extracts of both the plants (*A. indica* and *C. citratus*) was performed by inductively

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coupled plasma (ICP) spectroscopy. The obtained results are shown in Table 5.

Table 5. Metal Concentration (ppm) in Aqueous/Ethanolic Extracts of *A. indica* and *C. citratus*[a]

metal	concentration (ppm)			
	Eth extract of *A. indica* (EA)	Aq extract of *A. indica* (WA)	Eth extract of *C. citratus* (EC)	Aq extract of *C. citratus* (WC)
Al	1031±8.23	945±8.21	209±2.12	456±4.12
As	12±0.11	5±0.04	40±0.34	21±0.19
B	50±0.42	45±0.43	94±0.92	97±0.84
Ba	12±0.09	5±0.03	94±0.89	21±0.19
Ca	3000±28.1	3615±13.23	3675±13.54	7858±7.63
Cd	6±0.04	5±0.04	6±0.05	10±0.09
Co	6±0.05	5±0.04	33±0.31	10±0.01
Cr	93±0.87	25±0.21	87±0.76	43±0.23
Cu	68±0.53	105±1.13	358±3.02	326±2.12
Fe	843±7.21	230±2.12	351±3.21	652±2.23
K	13662±32.2	22345±22.23	39655±31.23	53750±6.45
Mg	2243±19.21	8375±16.21	2675±18.21	9956±9.23
Mn	12±0.11	35±0.32	27±0.23	152±1.23
Mo	12±0.09	15±0.12	20±0.19	32±0.21
Na	3181±12.23	1410±12.21	4925±23.21	8445±8.34
Ni	25±0.18	10±0.09	40±0.31	32±0.23
Pb	6±0.05	5±0.04	6±0.05	10±0.09
Sb	18±0.14	15±0.14	20±0.19	32±0.23
Se	18±0.12	15±0.10	20±0.17	32±0.21
Si	12±0.09	15±0.12	128±1.21	413±3.78
Sn	43±0.41	15±0.13	20±0.18	76±0.67
Sr	25±0.23	110±1.04	27±0.21	10±0.01
Ti	18±0.17	15±0.12	27±0.22	32±0.29
V	18±0.15	65±0.54	27±0.20	847±6.21
Zn	206±2.01	80±0.72	162±1.34	510±4.21
Zr	31±0.28	25±0.21	33±0.21	65±0.62

[a]The values were the average of triplicate samples (n = 3) ± S.D., two way ANOVA shows (P ≤ 0.05), so results are significant; The highest and lowest concentrations of each metal in the same row are highlighted by blue and green, font respectively.

[a]The values were the average of triplicate samples (n = 3) ± S.D., two-way ANOVA shows ($P \leq 0.05$), so results are significant; The highest and lowest concentrations of each metal in the same row are highlighted by blue and green, font respectively.

The data in Table 5 show that all leaf extracts (*A. indica* and *C. citratus*) contained Al, As, B, Ba, Ca, Cd, Cr, Cu, Co, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Sn, Sr, Ti, V, Zn, and Zr metals. The comparison of metal concentration in both plants indicates that *C. citratus* was generally richer in most of the metals (As, B, Ba, Ca, Cd, Cu, Co, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Sn, Ti, V, Zn, and Zr) as compared to *A. indica* (Table 5, the values of higher concentrations are highlighted in blue font). In *A. indica*, only four metals namely Al, Cr, Fe and Sr were present in higher concentration (values highlighted blue in Table 5) as compared to *C. citratus*; these four metals were found present only in ethanolic extract of former. The aqueous extract of *A. indica* had shown only the higher concentration of strontium as compared to remaining metals.

As far as the comparison between all four extracts is concerned (Table 5), *C. citratus* aqueous extract (WC) has generally shown higher concentrations of most of the investigated metals (i.e., B, Ca, K, Mg, Mn, Mo, Na, Pb, Sb, Se, Si, Sn, Ti, V, Zn, and Zr) as compared to all other investigated extracts (EA, WA, and EC). Lowest concentrations (values highlighted green in Table 5) of most of the metals (As, B, Ba, Cd, Co, Cr, Fe, Na, Ni, Pb, Sb, Se, Si, Sn, Ti, Zn, and Zr) were shown by the aqueous extract (WA) of *A. indica*, whereas its ethanolic extract (EA) has also shown the lowest concentrations of Ca, Cu, K, Mg, Mn, Mo, and V as compared to the remaining extracts. The lowest concentrations of Al and Sr were observed in *C. citratus* ethanolic and aqueous extracts, respectively, as compared to all the other extracts. *A. indica* (ethanolic extract) was enriched with high concentration of aluminum as compared to other extracts; the smallest amount of Al was found in *C. citratus* ethanolic extract. The lowest quantity of arsenic was present in *A. indica* aqueous (WA) and highest in *C. citratus* ethanolic extract (EC).

When we compare the concentration of different metals in the same extract, then the results are very interesting. Each of the ethanolic or aqueous extracts of any plant (*A. indica* or *C. citratus*) has shown the highest concentrations of potassium as compared to all other metals. However, the aqueous extract of *C. citratus* was richer in potassium (53,750 ppm) as compared to all other extracts (Table 5). All the investigated extracts (EA, WA, EC, and WC) generally contained higher concentrations of calcium, potassium, and sodium as compared to all other metals so all the extracts can be used as food supplements to fulfill the deficiencies of calcium, potassium, and sodium. Since the aqueous extract of *C. citratus* was richest in these three metals (Ca, K, Na) as compared to the other extracts, so it may be considered as the best food supplement for Ca, K, and Na. It is worth mentioning that all the investigated phytoextracts (EA, WA, EC, and WC) are nontoxic for human use as indicated by occurrence of their hemolytic values in the safe range (discussed in the Hemolytic Activity section).

Antioxidant Activity by the DPPH Method. Plants are commonly investigated for their antioxidant potential[76,77] since plant-derived antioxidants have the ability to inhibit oxidative stress and thus protect the nervous system from aging[78] and age-related diseases.[79] The antioxidant studies of the investigated plant extracts were performed by the DPPH method which is a quick method and involves free radical scavenging mechanism to determine the antioxidant activity. BHT (butylated hydroxytoluene) was used as a standard during these studies.

It was found that the ethanolic extracts of both plants are richer in antioxidant components. However, the ethanolic extract of *C. citratus* showed comparatively higher scavenging activity (74.50 ± 0.66%) as compared to that (70.48 ± 0.83%) of *A. indica* ethanolic extract. The higher antioxidant activity of the ethanolic extract is associated with its higher penetration power; the same kinds of results were also reported by other authors.[80,81] The aqueous extracts of both the plant leaves were comparatively less antioxidant as compared to ethanolic extracts. The aqueous extract of *C. citratus* displayed 67 ± 0.51% scavenging potential. However, only 32.22 ± 0.28% scavenging was exhibited by the aqueous extract of *A. indica* as shown in Table 6.

Synergistic Antibacterial Potential. Synergistic antibacterial potential of *A. indica* and *C. citratus* leaf extracts was evaluated by the biofilm inhibition method. The pure solutions (E_0, E_{00}, W_0, W_{00}) of ethanolic/aqueous extracts (EA, EC, WA,

Table 6. Radical Scavenging Activity of Leaf Extracts (*A. indica* and *C. citratus*)[a]

sample	radical scavenging (%)
ethanolic extract of *C. citratus* (EC)	74.50 ± 0.66
aqueous extract of *C. citratus* (WC)	67.00 ± 0.51
ethanolic extract of *A. indica* (EA)	70.48 ± 0.83
aqueous extract of *A. indica* (WA)	32.22 ± 0.28
BHT Standard	83.54 ± 0.91

[a]The values were the average of triplicate samples (n = 3) ± S.D.; One-way ANOVA shows ($P \leq 0.05$) so results are significant.

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Table 7. Synergistic Antimicrobial Potential (Biofilm Inhibition %) Data of Various Mixtures of Ethanolic and Aqueous Extracts of *A. indica* and *C. citratus*[a]

ethanolic extracts			aqueous extracts		
sample code	*S. aureus*	*E. coli*	sample code	*S. aureus*	*E. coli*
E_0	45.45 ± 0.38	43.75 ± 0.42	W_0	34.54 ± 0.32	9.37 ± 0.08
E_1	36.36 ± 0.31	28.13 ± 0.21	W_1	27.27 ± 0.28	no activity
E_2	20 ± 0.21	37.5 ± 0.32	W_2	21.81 ± 0.19	31.25 ± 0.30
E_3	38.18 ± 0.32	No activity	W_3	40.00 ± 0.35	no activity
E_4	38.18 ± 0.41	12.5 ± 0.11	W_4	43.63 ± 0.41	no activity
E_5	43.63 ± 0.52	15.63 ± 0.15	W_5	36.81 ± 0.29	no activity
E_6	25.45 ± 0.21	6.25 ± 0.05	W_6	50.90 ± 0.51	no activity
E_7	45.46 ± 0.42	9.38 ± 0.09	W_7	14.54 ± 0.12	no activity
E_8	36.36 ± 0.31	18.75 ± 0.15	W_8	3.63 ± 0.02	no activity
E_9	14.55 ± 0.12	18.75 ± 0.13	W_9	no activity	no activity
E_{00}	50.91 ± 0.42	59.38 ± 0.62	W_{00}	23.63 ± 0.21	6.25 ± 0.051
ciprofloxacin	47.27 ± 0.56	53.12 ± 0.48	ciprofloxacin	47.27 ± 0.39	53.12 ± 0.45

[a]The values were the average of triplicate samples (n = 3) ± S.D. One-way ANOVA shows ($P \leq 0.05$), so results are significant; E_0 = Solution containing 0.3 g of EA in 1 mL of ethanol; E_{00} = Solution containing 0.3 g of EC in 1 mL of ethanol; W_0 = Solution containing 0.3 g of WA in 1 mL of water; W_{00} = Solution containing 0.3 g of WC in 1 mL of water; E_1-E_9 are solutions of E_0 and E_{00} in 9:1, 8:2. 7:3, 6;4, 5:5, 4:6, 3:7, 2:8, and 1:9 ratios (V/V), respectively; W_1-W_9 are solutions of W_0 and W_{00} in 9:1, 8:2. 7:3, 6;4, 5:5, 4:6, 3:7, 2:8, and 1:9 ratios (V/V), respectively.

WC) and their mixtures (E_1-E_9 and W_1-W_9) were tested for their potential against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) by using ciprofloxacin as a standard antibacterial drug. The obtained results are shown in Table 7.

A comparison of data in Table 7 clearly shows that the pure plant extracts (E_0, E_{00}, W_0 and W_{00}) as well as their mixtures (E_1-E_9 and W_1-W_9) are generally more effective against *S. aureus* as compared to *E. coli*; it is due to the differences in the structures and strength of cell envelope of both bacteria. The cell wall of Gram-negative bacteria is more complex as compared to that of Gram-positive bacteria.[82] Moreover, the ethanolic extracts (E_{00}) of *C. citratus* were more active as compared to those (E_0) of *A. indica,* whereas the aqueous extracts (W_{00}) of *C. citratus* have shown lower activities as compared to those (W_0) of *A. indica*. However, the aqueous extract of each plant (*C. citratus* or *A. indica*) exhibited less bacterial inhibition as compared to its corresponding ethanolic extracts (Table 7). The aqueous extracts of *A. indica* (W_0) and *C. citratus* (W_{00}) have shown very little biofilm inhibitions of 9.37 and 6.25%, respectively, against *E. coli*. On the other hand, E_{00} (containing ethanolic extract of *C. citratus*) was found more potent among all the test solutions; it displayed 59.38% biofilm inhibition of *E. coli* and 50.91% that of *S. aureus*; these activities were found even higher as compared to those of standard drug namely ciprofloxacin (53.12% against *E. coli* and 47.27% against *S. aureus*).

The E_1-E_9 (mixtures of ethanolic extracts) demonstrated lower bacterial inhibition (antagonistic effect) as compared to their individual components (E_0-E_{00}) against each bacterium (*S. aureus* and *E. coli*). On the other hand, the W_3 to W_6 mixtures of aqueous extracts of both plants have shown higher bacterial inhibition (36.81−50.90%, synergistic effect) against *S. aureus* as compared to their individual components (W_0, 34.54%; $W_{00,}$ 23.63%). Against *E. coli*, only one aqueous mixture (W_2) has shown fantastic synergistic potential (31.25%) though its components W_0 (9.37%) and W_{00} (6.25%) have shown very little inhibition of the same bacterium (*E. coli*). Generally, the W_1-W_8 mixtures have shown significant bacterial inhibition against *S. aureus*, but they were found inactive against *E. coli* with few exceptions. The W_9 mixture was totally inactive against *E. coli* and *S. aureus*. The standard drug (ciprofloxacin) was found more potent (47.27% against *S. aureus* and 53.12% against *E. coli*) as compared to W_1-W_9 mixtures and their individual components (W_0 and W_{00}).

It is extremely difficult to compare the results of antibacterial screening with those reported earlier due to different strains assayed and numerous methodologies used.[83,84] However, in the light of previous studies on *A. indica*[85−88] and *C. citratus*,[25,89−91] it can be suggested that the investigated plant extracts and their mixtures have shown good activities against the tested microbes. Earlier studies report that the leaf extract of *A. indica* is composed of pharmacologically active constituents which induce activity against a variety of bacteria (e.g., *S. typhi*, *E. coli*, *S. aureus*, *P. aeruginosa*) and thus cause its usefulness in the treatment of various infectious diseases.[85] Its extracts are suitable for the treatment of various infections caused by *E. coli*, *S. aureus*, *K. ozanae,* and *P. aeruginosa*.[87] Its oil inhibits the synthesis of bacterial cell membrane and was found responsible for the killing of many pathogenic bacteria; its efficiency was much better at 4 °C as compared to 37 °C.[86] It was also reported that the antibacterial potential of *A. indica* may vary depending upon the plant parts (leaves, bark etc.) used, solvent medium for extraction, concentration of plant extracts and even the state of material (dry or fresh); the fresh extracts of *A. indica* barks/leaves are generally more potent.[88] *C. citratus* (lemongrass) also contains many secondary active metabolites which are associated with its pharmacological potential. The usefulness of its extracts against antibiotic resistant microorganisms has been suggested. Its extracts have shown activities against *Vibrio cholera*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Lysinibacillus macrolides,* and *Bacillus vallismortis* and displayed highest activity against *S. aureus*;[89] these findings are thus similar to those of our current studies that plant extracts and their mixtures are more effective against *S. aureus* as compared to *E. coli*. The water, methanol, and chloroform extracts *of C. citratus* leaves have also shown excellent potential against *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Bacillus subtilis,* and various health benefits are associated with this plant.[90] Its oil is more effective against Gram-positive bacteria as compared to Gram-negative bacteria and can be applied for the treatment of infections caused by

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multidrug resistant organisms.[25] Another study demonstrated the higher activity (against *Staphylococcus epidermis* and *Streptococcus mutans*) of lemongrass essential oil as compared to tetracycline and usefulness of its oil as an alternative of tetracycline or adjunctive for the treatment of periodontitis.[91]

Hemolytic Activities. Hemolytic activities were performed on human red blood cells to evaluate the toxic hemolytic effects of the investigated plant extracts and to find whether a compound is safe for future medicinal uses. Despite the plant's powerful antioxidant and antibacterial properties, its medical usage for human beings will be limited in the presence of hemolytic effects (more than 10%) and such a compound/extract cannot be adopted as a medicine by human beings. The results of activities are shown in Table 8.

Table 8. Hemolytic Activity Data of Aqueous and Ethanol Extracts (*C. citratus* and *A. indica*)[a]

sample	% of RBCs lysis
ethanolic extract of *C. citratus* (EC)	4.90 ± 0.003
aqueous extract of *C. citratus* (WC)	2.02 ± 0.001
ethanolic extract of *A. indica* (EA)	4.52 ± 0.005
aqueous extract of *A. indica* (WA)	2.88 ± 0.002
Triton X 100(positive control)	98.61 ± 0.83

[a]The values in Table 8 were the average of triplicate samples ($n = 3$) ± S.D.; One-way ANOVA shows $P \geq 0.05$, so results are nonsignificant. In hemolytic activity, nonsignificant is better because samples show less toxicity due to this less difference among sample to sample.

It was found that the investigated leaf extracts of *A. indica* and *C. citratus* have shown only very little hemolytic effects on red blood cells as compared to Triton X-100 (100% lysis)[92] and phosphate buffer solution (0% lysis). The ethanolic extracts of *C. citratus* and *A. indica* have shown hemolytic activities of 4.90 and 4.52%, respectively; these effects were further lowered in aqueous extracts to 2.02 and 2.88% in *C. citratus* and *A. indica*, respectively. The slightly large values in case of ethanolic extracts may be rendered to the effects of medium solvent (ethanol) used for the leaf extraction because ethanol depending upon its concentration may cause hemolysis by forming membrane pores in red blood cells due to which the cells finally swell and burst through a colloid-osmotic mechanism.[93] However, all the extracts have displayed hemolytic activities in the range of 2.02−4.90% indicating their possible safe use for human beings.[94]

EXPERIMENTAL SECTION

Sample Collection and Preparation. The leaves of *A. indica* and *C. citratus* were freshly collected from Raib, Kalachechi village of district Narowal (Punjab, Pakistan).

Preparation of Plant Extracts. Distilled water and analytical-grade ethanol were used for plant extraction due to their polar nature. Earlier studies show that ethanol and water solvents are more effective in extracting phenolic compounds due to their polar nature.[81]

Fresh leaves of *Azadirachta indica* and *cymbopogon citratus* (collected from Kalachechi village of Raib in Narowal, Pakistan). The leaves were dried in the shade and in a clean environment, cut into small pieces, and finally crushed into fine powder by a grinder machine.

Twenty-gram leaf powder of a plant (*A. indica* or *C. citratus*) was taken in a 250 mL conical flask, and then 100 mL of water was added into it. The flask was covered with aluminum foil. The mixture was then stirred in an orbital shaker for 24 h, filtered, and evaporated to leave behind the dried aqueous extracts WA and WC of *A. indica* and *C. citratus*, respectively. The same procedure was used to prepare the dried ethanolic extracts EA and EC of *A. indica* and *C. citratus*, respectively by using ethanol solvent. All the extracts (WA, WC, EA, and EC) were stored at 4 °C in a refrigerator for further analysis and use.

FTIR Spectroscopy. FTIR spectra of the solid products were recorded in the range of 4000−600 cm^{-1} by a Cary 630 FTIR spectrometer using Germanium ATR.

Determination of Total Phenolic and Total Flavonoid Contents. The Folins−Ciocalteu reagent method[95] was followed to determine the total phenolic contents. The reported procedure was used to determine the total flavonoid content.[96]

HPLC Analysis for Quantitative Determination of Phenolic and Flavonoid Contents. For HPLC analysis, fresh plant extracts were prepared (as filtrate) in ethanol/water and used without drying. Five milliliters of an extract (aqueous/ethanolic) was taken and centrifuged for 20 min at 4000rps. The upper liquid layer was decanted and again filtered through a 0.45 μm syringe filter for analysis. The HPLC (model 1260 Agilent, USA), equipped with quaternary pump 1260 and a DAD detector, was used. For data analysis, CHEMSTATION software was used. A 20 μL volume of the filtered sample was injected into a Zorbax Eclips Plus (Agilent, USA) reverse phase (C18) column (4.6 × 250 mm; 5 μm particle size).

For phenolic acid analysis, the mobile phase used consisted of a mixture of distilled methanol (solvent A) and 1% acetic acid (solvent B). The flow rate was 1 mL/min in a linear gradient following the scheme (t in min; %A): (0; 60%), (5; 35%), (10; 10%), (15, 60%), and (20; 60%). The column temperature was maintained at 25 °C. The chromatograms were recorded at 280 nm.[97]

For flavonoid analysis, two solvent systems A and B were used as mobile phase; A consisted of 3% trifluoroacetic acid while B contained acetonitrile and methanol (80:20 v/v). The chromatographic separation was performed by isocratic elution of the mobile phase (mixture of solvent A and B (50:50 v/v) that was filtered under vacuum through a 0.45 μm membrane before use) at a flow rate of 1 mL/ min at 30 °C. Detection was performed at a wavelength of 360 nm.[98]

Identification of phenolics and flavonoids was carried out by comparing their retention times with those of reference standards (Sigma Chemicals Co., St Louis, MO, USA). Quantitative determination was carried using calibration curves of the standards.

Sample Preparation for GC−MS Analysis. For GC−MS analysis, the methanolic solutions of plant extracts were used. The apparatus used for the GC−MS analysis consisted of a capillary column (30 m × 0.25 mm × 0.25 μm) with an electron ray of 70 eV. Oven temperature was programmed in a three step gradient: initial temp set at 45 °C (held for 5 min), ramped till 150 °C at 10 °C/min followed by a 5 °C/min rise till 280 °C and finally at 15 °C/min to 325 °C where it was held for 5 min. Helium gas flow rate was 1.1 mL/min (pressure 60 KPa and linear velocity 38.2 cm/s). Ions/fragments were monitored in scanning mode through 40−550 *m/z*. To compute the comparative percentage of each component, the

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assessment was done between its average climax regions to the total area.[99]

Metal Analysis by ICP. Two grams of a plant sample was taken in a Teflon vessel and then, 5 mL of $HClO_4$ and 2 mL of HNO_3 were added. The mixture was run in a microwave digester at 100 °C for 10 min and then at 180 °C for 15 min. The contents were cooled to room temperature followed by rinsing of vessel walls with grade-1 water, and the mixture was filtered into a 10 mL volumetric flask. The final filtrate was used for metal analysis by ICP spectroscopy using an ICP-OES (Agilent 720) instrument, whereas argon gas was used for the plasma source at 10,000 K. The concentration of metals was determined at various wavelengths of 237 nm (Al), 188 nm (As), 249 nm (B), 493 nm (Ba), 393 nm (Ca), 214 nm (Cd), 228 nm (Co), 205 nm (Cr), 324 nm (Cu), 259 nm (Fe), 769 nm (K), 280 nm (Mg), 257 nm (Mn), 204 nm (Mo), 589 nm (Na), 231 nm (Ni), 217 nm (Pb), 217 nm (Sb), 196 nm (Se), 288 nm (Si), 284 nm (Sn), 421 nm (Sr), 334 nm (Ti), 292 nm(V), 213 nm (Zn), and 343 nm (Zr).

Antioxidant Activities by DPPH Free Radical Scavenging Assay. DPPH (1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of the extracts was calculated using a reported method.[100]

$$\text{Scavenging (\%)} = 100 \times (A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}$$

where A is absorbance.

Synergistic Antibacterial Activities. The synergistic antibacterial activities of the aqueous and ethanolic extracts were evaluated by using the biofilm inhibition method reported earlier.[101,102] Pure solutions (E_0, E_{00}, W_0 and W_{00}; Table 9) of the investigated plant extracts (EA, EC, WA, and WC) and their mixtures (E_1-E_9 and W_1-W_9; Table 9) were evaluated for their synergistic antimicrobial activities against *S. aureus* and *E. coli* by using ciprofloxacin as a standard antibacterial drug. Nutrient broth was used as the growth medium in sterile 96-well flat-bottomed plastic tissue culture plates. The OD of each well was measured at 630 nm using a microplate reader (BioTek, USA).[103] The bacterial growth inhibition (INH%) was calculated as follows:

$$\text{INH\%} = 100 - (\text{OD}_{630\text{sample}} \times 100)/\text{OD}_{630\text{control}}$$

The preparation of the test solutions has been discussed below (under next heading).

Table 9. Preparations of Test Solutions of Plant Extracts of *A. indica* and *C. citratus*[a]

mixtures (E1-E9) of ethanolic solutions (E_0 and E_{00}) of dried ethanolic extracts (EA and EC) of *A. indica* and *C. citratus*			mixtures (W1-W9) of aqueous solutions (W_0 and W_{00}) of dried aqueous extracts (WA and WC) of *A. indica* and *C. citratus*		
code of mixture	mL of E_0	mL of E_{00}	code of mixture	mL of W_0	mL of W_{00}
E_1	0.9	0.1	W_1	0.9	0.1
E_2	0.8	0.2	W_2	0.8	0.2
E_3	0.7	0.3	W_3	0.7	0.3
E_4	0.6	0.4	W_4	0.6	0.4
E_5	0.5	0.5	W_5	0.5	0.5
E_6	0.4	0.6	W_6	0.4	0.6
E_7	0.3	0.7	W_7	0.3	0.7
E_8	0.2	0.8	W_8	0.2	0.8
E_9	0.1	0.9	W_9	0.1	0.9

[a]EA = Ethanolic extract (dried) of *A. indica*; EC = Ethanolic extract (dried) of *C. citratus*; WA = Water extract (dried) of *A. indica*; WC = Water extract (dried) of *C. citratus*; E_0 = Solution containing 0.3 g of EA in 1 mL of ethanol; E_{00} = Solution containing 0.3 g of EC in 1 mL of ethanol; W_0 = Solution containing 0.3 g of WA in 1 mL of water; W_{00} = Solution containing 0.3 g of WC in 1 mL of water.

Preparation of Solutions for Synergistic Antibacterial Screening. Four extract solutions (E_0/E_{00}/W_0/W_{00}) were obtained by dissolving 0.3 g of a dried plant extract (EA/EC/WA/WC) in 1 mL solvent (ethanol or water). Solutions E_0 and E_{00} were obtained by dissolving 0.3 g of EA and EC, respectively, in 1 mL of ethanol. Similarly, solutions W_0 and W_{00} were prepared by mixing 0.3 g of WA and WC, respectively, in 1 mL of water.

Finally, E_0 and E_{00} were mixed with each other in 9:1, 8:2. 7:3, 6;4, 5:5, 4:6, 3:7, 2:8, and 1:9 ratios (V/V) to produce the test solutions E_1-E_9, respectively (Table 9), whereas mixing of W_0 and W_{00} in the same ratios (9:1, 8:2. 7:3, 6;4, 5:5, 4:6, 3:7, 2:8, and 1:9) was used to prepare the test solutions W_1-W_9, respectively (Table 9).

Hemolytic Activities. Hemolytic activity evaluations of the plant extracts (EC, WC, EA, and WA) were performed on human red blood cells by a reported procedure using Triton X100 as a positive control and phosphate buffer as a negative control.[104,105]

Statistical Analysis. All the experiments in the present research work were conducted three times (n = 3) unless stated otherwise, and statistical analysis of the data was performed by analysis of variance.[106,107]

CONCLUSIONS

The ethanolic and aqueous extracts of *A. indica* and *C. citratus* leaves have shown the presence of alcoholic, carboxylic, aldehydic, halo, and alkenic functional groups. Total flavonoids in ethanolic extracts were present in higher quantities (90.56−100.56 mg CE/100 g) as compared to those (22.69−23.76 mg CE/100 g) in aqueous extracts of plants. The ethanolic extracts were also richer (65.70−131.98 mg GAE/100 g) in total phenolics as compared to those (14.53−38.95 mg GAE/100 g) in water extracts. The ethanolic extract of *C. citratus* contained higher concentrations of caffeic acid (1.432 mg/g), synapic acid (6.743 mg/g), and benzoic acid (7.431 mg/g) as compared to all other extracts, whereas chlorogenic acid (0.311 mg/g) was present only in the aqueous extract of *A. indica*. The ethanolic extract of *C. citratus* can be used as a food preservative as it is richer in benzoic acid (7.431 mg/g). GC−MS analysis demonstrated the presence of 36 and 23 compounds in *A. indica* and *C. citratus* leaves, respectively. The extracts were generally richer in calcium (3000−7858 ppm), potassium (13662−53,750 ppm), and sodium (3181−8445 ppm) and hence can be used in food supplements as a source of these metals, the aqueous extract of *C. citratus* being the best in this regard. Antioxidant potential (DDPH method) of *C. citratus* ethanolic extract was the highest (74.50 ± 0.66%), whereas it was lowest (32.22 ± 0.28%) for the aqueous extract of *A. indica*. The plant extracts and their mixtures were more active against *S. aureus* as compared to *E. coli*. The ethanolic extract of *C. citratus* have displayed the highest biofilm inhibition of *E. coli* (59.38%) and *S. aureus* (50.91%) as compared to all the other test solutions. The antibacterial activities were lowered (antagonistic effect) when ethanolic extracts of both the plants were mixed together.

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However, biofilm inhibition was increased (synergistic effect) when aqueous extracts of *A. indica* and *C. citratus* were mixed together in certain specific ratios. All the extracts have displayed hemolytic activities in the range of 2.02−4.90% indicating their possible safe use for human beings.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c06785.

FTIR spectra of EA, WA, EC, and WC (Figures S1−S4); their HPLC chromatograms (Figures S5−S8 for phenolics) and (S9−S12 for flavonoids) and their GCMS spectra (Figures S13−S16) (PDF)

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Notes

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