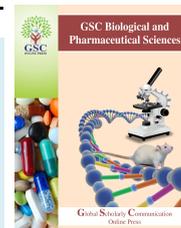


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GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



Phytochemicals and phyto-disinfectant properties of citrus species (*Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis*) for pond water purification

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Publication history: Received on 27 July 2019; revised on 12 August 2019; accepted on 14 August 2019

Article DOI: <https://doi.org/10.30574/gscbps.2019.8.2.0139>

Abstract

The health concern associated with the use of chemical disinfectant in water purification has necessitated the search of safe and effective water treatment agents from natural sources. The leaf, stem, seed and bark of *Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis* were investigated for phytochemicals and disinfectant properties on contaminated pond water at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 g/L. Microbial quality of the water sample was investigated at 12 hrs interval. The leaf and bark of *C. aurantifolia* and the bark of *C. limon* contains all the phytochemicals tested. Steroids and alkaloids were present in all parts of the plant. Cardiac glycosides and terpenes were present in all parts of *Citrus sinensis*. A total of 10 different bacteria isolates were identified from ponds water sample. The stem of *Citrus aurantifolia* completely eliminated the total viable count, total coliform count, fecal coliform count after 12 h. The leaf, bark and seed of *C. aurantifolia* at 0.3, 0.4 and 0.5 g/L, leaf and bark of *C. limon* and *C. sinensis* at 0.1-0.5 g/L eliminated the various bacteria after 24 hours. However, the stem and seed of *C. limon* were less active; only concentrations of 0.4 and 0.5g/L eliminated the organism after 24 h of treatment. The plant materials had minimum inhibitory concentration in the ranged of 16 and 128 mg/g and minimum bactericidal concentrations in the ranged 16 and 256 mg/g against all the isolates. It is concluded that the leaf, stem bark and seed of citrus species contains various phytochemicals and have the potentials to serve as a disinfectant for water treatment.

Keywords: Antimicrobial; *Citrus limon*; *Citrus aurantifolia*; *Citrus sinensis*; Drinking water; Phytochemicals

1. Introduction

Drinking water is indispensable for human existence, it is used to meet up the basic human need including food, shelter and clothing, thus serve as essential ingredients for wellbeing and a healthy life [1]. Some rural and urban area of Nigeria suffers a severe drinking water supply crisis, particularly in the dry seasons of every year. The drinking water supplies in the cities is intermittent. Nearly all of the surface sources and ground water sources have been exploited. The growing imbalance between supply and demand has led to chronic shortages and competition that have resulted in pollution and environmental degradation. Apart from quantitative shortages, the quality of drinking water in the rural and urban area of the country is becoming a serious public health issue for the past few years [2]. The quality of water for drinking has deteriorated because of the treatment inadequacy, direct discharge of untreated sewage into rivers, ponds and inefficient management of the piped water distribution system [3].

Diarrhoea, typhoid fever, and cholera are caused by poor sanitation, hygiene and water quality, are one of the most prevalent water borne disease in Nigeria. Ideally, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution [4]. The world health organization has estimated that up to 80% of all diseases and sickness in the world is caused by inadequate sanitation, polluted water or unavailability of water

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however, 10% could be prevented by improvements related to drinking water, sanitation, hygiene and water resource management [5]. Larger populations of Nigerians depend on raw water for drinking purposes without any treatment whatsoever.

Africa is endowed with large amounts of medicinal plants with important source of active ingredients which differ widely in terms of structure and therapeutic properties [6-8]. The importance of plants as antimicrobial remains of greater relevance with the current global shift to obtain remedies from plants sources owing to their safety, efficacy and availability [9-10]

Indigenous knowledge indicates that there are several plant species that can be used as a water disinfectant [11]. Recent studies have also indicated the efficiency of various plants seed on surface water purification [11-12]. However, less attention has been placed on leaf stem and root of plant. Furthermore, no adequate attention has been given to members of the genus citrus. Citrus is the largest genus belong to the family Rutaceae, it is one of the most essential commercial fruit crops which are grown in all continents of the world [13]. Although sweet orange (Citrus) is the major fruit in this group accounting for about 70% of citrus output, the group also encompasses small citrus fruits such as *Citrus sinensis*, lime tree (*Citrus aurantifolia*) and lemon tree (*Citrus limonum*) [14]. A large number of plants have been investigated scientifically for antimicrobial activity and a number of citrus plants are rich source of phytochemicals and have been shown to inhibit the growth of pathogenic microorganism [15]. Thus, these plants could serve as a natural disinfectant which are environment friendly and with much reduced risk of handling. The present study therefore, screened *Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis*) for phytochemical composition and their disinfectant potential against microbial population in pond water.

2. Material and methods

2.1. Collection and identification of plant materials

Seeds, roots, bark and leaves of *Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis*, were collected from Bosso Local Government Area of Minna Niger State based on their uses in water purification and ethno medicine. The plants were identified at and authenticated at NIPRID Abuja where the vouchers numbers NIPRD/H/7024, NIPRD/H/7026 and NIPRD/H/7025 respectively were deposited.

2.2. Sample preparation and phytochemical analysis

The leaves, stem, bark and seed samples of *Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis* were washed and dried for 2 weeks (37°C) and finally grounded using a grinder mill. Quantitative phytochemical analysis of the plant was carried out using standard procedures as described previously [16-17].

2.3. Collection of water samples and identification of bacterial isolates

Pond water sample was collected from Chanchaga Local Government Area of Minna, Niger State using Standard sampling methods of APHA [18]. Characterization and identification on isolates was carried out adopting methods of Vandepitte et al. [19] and Cheesbrough [20].

2.4. Water purification

The powdered plant material was suspended in the pond water sample at concentrations of 0.1, 0.2, 0.3, 0.4 and 0.5 g/l. The treated water was covered, left to settle for an hour and then filtered [21]. The clean water from the solution was then taken after treatment for microbial analysis.

2.5. Bacteriological analysis of water

Membrane filtration technique was employed to determine microbial quality of the water samples in accordance with American Public Health Association (APHA). Total viable counts (TVC) were done by plating serially diluted water samples on nutrient agar. All plates were incubated at temperature of 37°C for 24 hrs. The total coliforms by plating on MacConkey agar, while the fecal coliforms count was performed using most probable number (MPN) method. A broth microdilution method [22], was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract in triplicates.

2.6. Statistical analysis

Data generated were analyzed using statistical package for social science (SPSS). Differences between groups were compared using analysis of variance, ANOVA ($P < 0.05$) followed by Duncan's Multiple Range Test.

3. Results

3.1. Qualitative phytochemical composition of plant used for water purification

The qualitative phytochemical composition of the seed, leaf, bark and stem of *Citrus limon*, *Citrus aurantifolia*, *Citrus sinensis*, are presented in Table 1: The leaf and bark of *Citrus aurantifolia* and the bark of *Citrus limon* contains all the phytochemicals tested. Steroids, alkaloids were present in all parts of the three plant. Anthraquinone was present in all parts of *Citrus limon* and absent in all part of *Citrus sinensis*. Cardiac glycosides and terpenes were present in all part of *Citrus sinensis*. Phenol, flavonoids and tannins were present in leaf, bark and stem of *Citrus aurantifolia*, Phenol, flavonoids, saponins, tannins, Cardiac glycosides and anthraquinone were absent in seed of *Citrus aurantifolia*.

3.2. Biochemical characteristic and frequency of occurrence of the Bacterial isolates

A total of 10 different bacteria isolates including; *Eschecherichia coli*, *Shigella dysenteriae*, *Salmonella* spp, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus megatarium*, *Pseudomonas aeruginosa*, *Kluyvera ascorbate*, *Proteus myxofaciens* and *Enterobacter aerogenes* were identified from ponds water samples.

Table 1 Phytochemical composition of citrus plant species used for water purification

Plant	Part	Phenols	Flavonoids	Saponin	Tannin	Alkaloids	Cardiac Glycosides	Anthraquinones	Terpenes	Steroid
<i>Citrus limon</i>	Seed	-	-	-	-	+	-	+	-	+
	Leaf	+	-	-	+	+	-	+	+	+
	Bark	+	+	+	+	+	+	+	+	+
	Stem	+	-	-	+	+	-	+	+	+
<i>Citrus aurantifolia</i>	Seed	-	-	-	-	+	-	-	+	+
	Leaf	+	+	-	+	+	+	+	+	+
	Bark	+	+	+	+	+	+	+	+	+
	Stem	+	+	+	+	+	-	-	-	+
<i>Citrus sinensis</i>	Seed	-	-	-	-	+	+	-	+	+
	Leaf	+	+	-	-	+	+	-	+	+
	Bark	-	+	-	+	+	+	-	+	+
	Stem	-	+	-	-	+	+	-	+	+

Key: + (Present), - (Absent)

3.3. Effect of citrus plants on bacteriological quality of pond water

The untreated pond water had the TVC of $6.10 \pm 0.04 \times 10^5$ cfu/ml, $3.30 \pm 0.56 \times 10^5$ cfu/ml and $3.30 \pm 0.03 \times 10^5$ cfu/ml at 0, 12 and 24 h respectively, TCC were $2.96 \pm 0.24 \times 10^3$ cfu/ml, $2.96 \pm 0.03 \times 10^3$ cfu/ml and $2.96 \pm 0.03 \times 10^3$ cfu/ml at 0, 12 and 24 h respectively, FCC were 250.34 ± 0.89 mpn/100ml, 250.24 ± 3.45 mpn/100ml and 250.56 ± 2.87 mpn/100ml at 0, 12 and 24 h respectively while SSC were $1.68 \pm 0.03 \times 10^3$ cfu/ml, $1.68 \pm 0.03 \times 10^3$ cfu/ml and $1.68 \pm 0.03 \times 10^3$ cfu/ml at 0, 12 and 24 h respectively (Table 2).

The stem, seed, leaf and bark materials of *Citrus aurantifolia* at concentrations of 0.3, 0.4 and 0.5 g/l produced a complete elimination of the bacteria from the pond water after 24 hours of treatment. Only the stem material produces a complete elimination of the organism at 12 h (table 2).

The leaf and bark materials of *Citrus limon* at all concentrations tested (0.1, 0.2, 0.3, 0.4 and 0.5 g/L) completely eliminated the various bacteria from of the pond water after 24 hours of treatment. However, the stem and seed were less active; only concentrations of 0.4 and 0.5g/L caused the elimination of the organism after 24h of treatment (Table 3).

Table 2 Bacterial counts obtained from treated pond water with *Citrus aurantifolia*

Sample (g/L)	TVC x10 ⁵ cfu/mL			TCC x10 ³ cfu/mL			FCC mpn/100 mL			SSC x10 ³ cfu/mL		
	0	12	24	0	12	24	0	12	24	0	12	24
Pond water	6.10±0.04	3.30±0.56	3.30±0.03	2.96±0.24	2.96±0.03	2.96±0.03	250.34±0.89	250.24±3.45	250.56±2.87	1.68±0.03	1.68±0.03	1.68±0.03
Leaf of <i>C. aurantifolia</i>												
0.1	6.10±0.03	1.03±0.02	Nil	3.30±0.03	0.39±0.02	Nil	243.45±0.52	6.43±0.98	Nil	1.68±0.03	0.82±0.42	Nil
0.2	6.10±0.03	0.89±0.02	Nil	3.30±0.03	Nil	Nil	242.54±0.43	2.89±0.94	Nil	1.68±0.34	0.02±0.34	Nil
0.3	6.10±0.03	0.62±0.02	Nil	3.30±0.03	Nil	Nil	232.54±0.45	Nil	Nil	1.68±0.90	Nil	Nil
0.4	6.10±0.03	0.09±0.02	Nil	3.30±0.03	Nil	Nil	230.32±0.32	Nil	Nil	1.68±0.63	Nil	Nil
0.5	6.10±0.03	Nil	Nil	3.30±0.03	Nil	Nil	224.87±0.45	Nil	Nil	1.68±0.90	Nil	Nil
Stem of <i>C. aurantifolia</i>												
0.1	6.10±0.03	1.43±0.02	1.01±0.02	3.30±0.03	0.59±0.02	0.25±0.02	235.45±0.54	7.42±0.69	Nil	1.68±0.03	0.55±0.34	0.25±0.23
0.2	6.10±0.03	1.00±0.02	0.58±0.02	3.30±0.03	0.32±0.02	Nil	235.43±0.36	1.97±0.98	Nil	1.68±0.78	0.32±0.34	Nil
0.3	6.10±0.03	0.53±0.02	Nil	3.30±0.03	Nil	Nil	231.43±0.65	Nil	Nil	1.68±0.43	Nil	Nil
0.4	6.10±0.03	0.45±0.02	Nil	3.30±0.03	Nil	Nil	229.76±0.54	Nil	Nil	1.68±0.03	Nil	Nil
0.5	6.10±0.03	0.33±0.02	Nil	3.30±0.03	Nil	Nil	225.45±0.65	Nil	Nil	1.68±0.89	Nil	Nil
Seed of <i>C. aurantifolia</i>												
0.1	6.10±0.03	1.22±0.02	1.00±0.02	3.30±0.03	0.76±0.02	0.52±0.02	230.34±0.39	13.89±0.45	5.45±0.67	1.68±0.63	0.92±0.34	0.50±0.34
0.2	6.10±0.03	0.93±0.02	0.63±0.02	3.30±0.03	0.62±0.02	0.43±0.02	228.54±0.89	11.45±0.39	1.07±0.65	1.68±0.54	0.52±0.92	0.41±0.34
0.3	6.10±0.03	0.61±0.02	Nil	3.30±0.03	0.39±0.02	Nil	223.67±0.34	6.56±0.89	Nil	1.68±0.90	Nil	Nil
0.4	6.10±0.03	0.36±0.02	Nil	3.30±0.03	0.39±0.02	Nil	219.54±0.65	2.45±0.09	Nil	1.68±0.32	Nil	Nil
0.5	6.10±0.03	0.07±0.02	Nil	3.30±0.03	Nil	Nil	210.93±0.89	Nil	Nil	1.68±0.94	Nil	Nil
Bark of <i>C. aurantifolia</i>												
0.1	6.10±0.03	0.99±0.02	Nil	3.30±0.03	0.70±0.02	Nil	245.78±0.84	6.45±0.78	Nil	1.68±0.43	0.21±0.02	Nil
0.2	6.10±0.03	0.82±0.02	Nil	3.30±0.03	0.67±0.02	Nil	243.43±0.45	Nil	Nil	1.68±0.54	Nil	Nil
0.3	6.10±0.03	0.69±0.02	Nil	3.30±0.03	0.32±0.02	Nil	241.43±0.98	Nil	Nil	1.68±0.43	Nil	Nil
0.4	6.10±0.03	0.45±0.02	Nil	3.30±0.03	0.10±0.02	Nil	222.24±0.98	Nil	Nil	1.68±0.89	Nil	Nil
0.5	6.10±0.03	0.29±0.02	Nil	3.30±0.03	Nil	Nil	220.56±0.93	Nil	Nil	1.68±0.54	Nil	Nil

Data are MEAN ± SEM of triplicate determination
TVC: Total viable counts, TCC; total coliforms count, FCC: faecal coliforms count; SSC: Salmonella-shigella count

Table 3 Bacterial counts obtained from treated pond with *Citrus limon*

Sample (g/L)	TVC x10 ⁵ cfu/mL		TCC x10 ³ cfu/mL		FCC mpn/100 mL		SSC x10 ³ cfu/mL					
	0	12	24	0	12	24	0	12	24			
Pond water	6.10±0.03	6.10±0.03	6.10±0.03	3.30±0.03	3.30±0.03	250.00±0.00	250.00±0.00	1.68±0.03	1.68±0.03	1.68±0.03		
Leaf of <i>C. limon</i>												
0.1	6.10±0.03	2.00±0.02	Nil	3.30±0.03	1.08±0.02	Nil	243.70±7.86	16.00±0.00	Nil	1.68±0.03	0.25±0.02	Nil
0.2	6.10±0.03	1.25±0.02	Nil	3.30±0.03	0.89±0.02	Nil	242.00±0.99	11.00±0.00	Nil	1.68±0.03	0.11±0.02	Nil
0.3	6.10±0.03	1.11±0.02	Nil	3.30±0.03	0.33±0.02	Nil	232.00±2.00	2.00±0.00	Nil	1.68±0.03	0.07±0.02	Nil
0.4	6.10±0.03	0.06±0.02	Nil	3.30±0.03	0.11±0.02	Nil	230.50±0.00	Nil	Nil	1.68±0.03	Nil	Nil
0.5	6.10±0.03	Nil	Nil	3.30±0.03	0.11±0.02	Nil	224.00±0.70	Nil	Nil	1.68±0.03	Nil	Nil
Stem of <i>C. limon</i>												
0.1	6.10±0.03	2.10±0.02	1.82±0.02	3.30±0.03	1.21±0.02	1.00±0.02	234.00±0.60	38.60±0.40	21	1.68±0.03	0.72±0.02	0.51±0.02
0.2	6.10±0.03	1.61±0.02	1.40±0.02	3.30±0.03	0.70±0.02	0.41±0.02	234.00±0.07	20.00±0.00	15	1.68±0.03	0.66±0.02	0.41±0.02
0.3	6.10±0.03	0.82±0.02	0.01±0.02	3.30±0.03	0.39±0.02	0.12±0.02	231.60±0.00	11.50.00±0.50	6	1.68±0.03	0.89±0.01	0.20±0.01
0.4	6.10±0.03	0.69±0.02	0.21±0.02	3.30±0.03	0.11±0.02	Nil	229.00±0.40	8.00±0.00	Nil	1.68±0.03	0.34±0.01	Nil
0.5	6.10±0.03	0.33±0.02	0.12±0.02	3.30±0.03	Nil	Nil	225.00±0.00	Nil	Nil	1.68±0.03	Nil	Nil
Seed of <i>C. limon</i>												
0.1	6.10±0.03	2.06±0.02	1.54±0.02	3.30±0.03	1.36±0.03	1.04±0.03	230.00±0.00	60.00±0.00	39	1.68±0.03	0.89±0.02	0.74±0.02
0.2	6.10±0.03	1.88±0.02	1.40±0.02	3.30±0.03	1.36±0.03	0.91±0.03	228.00±0.00	41.00±9.00	24	1.68±0.03	0.75±0.02	0.62±0.02
0.3	6.10±0.03	1.49±0.02	Nil	3.30±0.03	1.22±0.03	Nil	223.00±0.00	28.00±0.00	Nil	1.68±0.03	0.66±0.02	Nil
0.4	6.10±0.03	1.16±0.02	Nil	3.30±0.03	0.67±0.03	Nil	219.00±0.00	16.00±0.00	Nil	1.68±0.03	0.48±0.02	Nil
0.5	6.10±0.03	0.36±0.02	Nil	3.30±0.03	0.45±0.03	Nil	210.00±0.00	4.00±0.00	Nil	1.68±0.03	Nil	Nil
Bark of <i>C. limon</i>												
0.1	6.10±0.03	0.50±0.02	Nil	3.30±0.03	0.63±0.02	Nil	244.00±0.00	5.00±0.00	Nil	1.68±0.03	0.62±0.02	Nil
0.2	6.10±0.03	0.38±0.02	Nil	3.30±0.03	0.39±0.02	Nil	243.00±7.00	2.00±0.00	Nil	1.68±0.03	0.39±0.02	Nil
0.3	6.10±0.03	0.29±0.02	Nil	3.30±0.03	0.31±0.02	Nil	241.00±7.50	Nil	Nil	1.68±0.03	Nil	Nil
0.4	6.10±0.03	0.27±0.02	Nil	3.30±0.03	0.18±0.02	Nil	222.00±0.00	Nil	Nil	1.68±0.03	Nil	Nil
0.5	6.10±0.03	Nil	Nil	3.30±0.03	Nil	Nil	220.00±0.00	Nil	Nil	1.68±0.03	Nil	Nil

Data are MEAN ± SEM of triplicate determination.
 TVC: Total viable counts, TCC; total coliforms count, FCC: faecal coliforms count; SSC: Salmonella-shigella count

Table 4 Bacterial counts obtained from treated pond water with *Citrus sinensis*

Sample (g/L)	TVC x10 ⁵ cfu/mL			TCC x10 ³ cfu/mL			FCC mpn/100ml			SSC x10 ³ cfu/mL		
	0	12	24	0	12	24	0	12	24	0	12	24
Pond water	6.10±0.03	6.10±0.03	6.10±0.03	3.30±0.03	3.30±0.03	3.30±0.03	250.00±0.00	250.00±0.00	250.00±0.00	1.68±0.03	1.68±0.03	1.68±0.03
Leaf of <i>C. sinensis</i>												
0.1	6.10±0.03	2.12±0.02	Nil	3.30±0.03	2.03±0.02	Nil	243.50±0.60	22.05±0.00	Nil	1.68±0.03	0.54±0.02	Nil
0.2	6.10±0.03	1.84±0.02	Nil	3.30±0.03	1.62±0.02	Nil	239.00±0.30	19.00±0.50	Nil	1.68±0.03	0.42±0.02	Nil
0.3	6.10±0.03	1.24±0.02	Nil	3.30±0.03	0.88±0.02	Nil	232.30±0.00	8.00±0.00	Nil	1.68±0.03	0.22±0.02	Nil
0.4	6.10±0.03	0.88±0.02	Nil	3.30±0.03	0.36±0.02	Nil	230.00±0.20	Nil	Nil	1.68±0.03	0.09±0.02	Nil
0.5	6.10±0.03	0.62±0.02	Nil	3.30±0.03	0.18±0.02	Nil	224.50±0.06	Nil	Nil	1.68±0.03	Nil	Nil
Stem of <i>C. sinensis</i>												
0.1	6.10±0.03	2.33±0.02	2.01±0.02	3.30±0.03	1.33±0.02	1.00±0.02	234.00±0.00	52.05±0.00	38.00±0.46	1.68±0.03	0.96±0.02	0.44±0.02
0.2	6.10±0.03	2.03±0.02	1.84±0.02	3.30±0.03	1.29±0.02	0.57±0.02	234.50±0.20	23.00±0.00	15.00±0.43	1.68±0.03	0.66±0.02	0.34±0.02
0.3	6.10±0.03	1.44±0.02	1.14±0.02	3.30±0.03	1.09±0.02	0.42±0.02	231.00±0.00	18.02±0.30	9.00±0.00	1.68±0.03	0.42±0.02	0.23±0.02
0.4	6.10±0.03	1.43±0.02	0.85±0.02	3.30±0.03	0.36±0.02	0.11±0.02	229.30±0.40	18.00±0.00	7.00±0.00	1.68±0.03	0.13±0.02	0.01±0.02
0.5	6.10±0.03	1.06±0.02	Nil	3.30±0.03	0.20±0.02	Nil	221.00±0.00	7.00±0.05	Nil	1.68±0.03	Nil	Nil
Seed of <i>C. sinensis</i>												
0.1	6.10±0.03	2.84±0.03	2.51±0.03	3.30±0.03	1.89±0.03	1.51±0.03	229.00±0.50	89.00±0.00	69.00±0.55	1.68±0.03	0.90±0.02	0.74±0.02
0.2	6.10±0.03	2.66±0.03	2.32±0.03	3.30±0.03	1.42±0.03	1.12±0.03	228.00±0.00	52.00±0.78	47.00±0.00	1.68±0.03	0.85±0.02	0.61±0.02
0.3	6.10±0.03	2.66±0.03	2.12±0.03	3.30±0.03	1.32±0.03	0.45±0.03	222.00±0.20	37.00±0.00	28.00±0.43	1.68±0.03	0.83±0.02	0.52±0.02
0.4	6.10±0.03	2.24±0.03	1.91±0.03	3.30±0.03	0.69±0.03	0.22±0.03	219.00±0.50	22.00±0.24	15.00±0.00	1.68±0.03	0.33±0.02	0.12±0.02
0.5	6.10±0.03	1.98±0.03	Nil	3.30±0.03	0.42±0.03	Nil	210.00±0.20	13.00±0.00	Nil	1.68±0.03	0.21±0.02	Nil
Bark of <i>C. sinensis</i>												
0.1	6.10±0.03	1.21±0.02	Nil	3.30±0.03	1.16±0.02	Nil	243.00±0.00	9.00±0.65	Nil	1.68±0.03	0.48±0.02	Nil
0.2	6.10±0.03	1.06±0.02	Nil	3.30±0.03	0.92±0.02	Nil	243.00±0.56	6.00±0.22	Nil	1.68±0.03	0.32±0.02	Nil
0.3	6.10±0.03	0.48±0.02	Nil	3.30±0.03	0.33±0.01	Nil	241.00±0.00	Nil	Nil	1.68±0.03	0.27±0.02	Nil
0.4	6.10±0.03	0.43±0.02	Nil	3.30±0.03	Nil	Nil	222.00±0.35	Nil	Nil	1.68±0.03	Nil	Nil
0.5	6.10±0.03	Nil	Nil	3.30±0.03	Nil	Nil	220.00±0.36	Nil	Nil	1.68±0.03	Nil	Nil

TVC: Total viable counts, TCC; total coliforms count, FCC: faecal coliforms count, SSC: Salmonella-shigella count
Data are MEAN ± SEM of triplicate determination

Table 5 Minimum inhibitory concentrations (MIC) of the plant materials against the isolated organism

Plant	Part	Ec	Bm	Sd	Pa	Ea	S	Pm	Kp	Ka	Sa
<i>Citrus limon</i>	Seed	16	32	16	64	16	16	16	16	128	32
	Leaf	32	16	32	64	32	32	32	32	64	16
	Bark	16	32	16	64	16	16	16	16	16	16
	Stem	16	16	16	128	32	32	32	32	128	16
<i>Citrus aurantifolia</i>	Seed	16	16	-	16	16	16	16	16	16	16
	Leaf	32	32	-	32	16	32	32	32	16	16
	Bark	16	16	-	16	32	16	16	16	16	16
	Stem	16	16	-	32	16	32	16	32	16	32
<i>Citrus sinensis</i>	Seed	16	16	64	64	32	16	32	64	64	16
	Leaf	64	32	16	64	16	16	32	64	64	32
	Bark	64	64	16	16	32	16	16	16	16	16
	Stem	64	16	32	32	32	16	16	16	32	32

Ec- *E. coli*; Bm- *B. megaterium*; Sd- *S. dysenteriae*; Pa- *P. aeruginosa*; Ea- *E. aerogenes*; S- *Salmonella spp*; Pm- *P. myxofaciens*; Kp- *K. pneumoniae*; Ka- *K. ascorbata*; Sa- *S. aureus*

Table 6 Minimum bactericidal concentrations (MBC) of the plant materials against the isolated organism

Plant	Part	Ec	Bm	Sd	Pa	Ea	S	Pm	Kp	Ka	Sa
<i>Citrus limon</i>	Seed	32	32	32	64	32	16	16	16	128	32
	Leaf	64	16	64	128	256	32	32	64	64	16
	Bark	32	32	16	64	16	32	16	16	16	64
	Stem	32	16	16	128	32	32	32	32	256	32
<i>Citrus aurantifolia</i>	Seed	32	32	-	64	64	64	32	16	32	32
	Leaf	32	16	-	32	32	32	32	32	16	16
	Bark	32	16	-	16	32	16	16	16	16	16
	Stem	32	16	-	32	64	32	16	32	64	32
<i>Citrus sinensis</i>	Seed	16	16	128	64	32	32	32	64	64	16
	Leaf	64	32	64	64	16	32	32	64	64	32
	Bark	64	64	16	16	32	32	16	16	34	64
	Stem	64	16	128	32	32	64	16	16	32	32

Ec- *E. coli*; Bm- *B. megaterium*; Sd- *S. dysenteriae*; Pa- *P. aeruginosa*; Ea- *E. aerogenes*; S- *Salmonella spp*; Pm- *P. myxofaciens*; Kp- *K. pneumoniae*; Ka- *K. ascorbata*; Sa- *S. aureus*

The leaf and bark materials of *Citrus sinensis* at all concentrations tested (0.1, 0.2, 0.3, 0.4 and 0.5 g/L) completely eliminated the various bacteria from of the pond water after 24 hours of treatment. However, the stem and seed were less active; only concentrations of 0.5g/L caused the elimination of the organism after 24h of treatment (Table 4).

3.4. Minimum inhibitory concentrations (MIC) and Minimum bacterial concentrations (MBC)

The minimum inhibitory concentrations (MIC) of the citrus plant materials against the isolated organism are presented in table 5. The MIC range between 16 and 128 against all the isolates tested. *Citrus aurantifolia* materials were inactive against *S. dysenteriae*. The minimum bactericidal concentrations (MIC) of *Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis* material (seed, leaf, bark and stem) against the isolated organism are range between 16 and 256 against all the isolates tested (table 6).

4. Discussion

Plants constitute an important source of active ingredients known as secondary metabolite which confers to them some physical characteristic and also defense purposes [23]. However, these metabolites have been reported for various beneficial properties to humanity [24]. The photochemical screening of citrus plants indicated the presence various phytochemicals which have been implicated in various biological activities [24-25]

The phytochemical composition observed in leaf, stem, seed and bark (Table 1) of Citrus species (*Citrus limon*, *Citrus aurantifolia*, *Citrus sinensis*) is in line with previous studies which reported that citrus plants synthesize and accumulate in their cells a great variety of phytochemicals including low molecular phenolic (hydroxy benzoic and hydroxycinnamic acids, acetophenones, terpenoids, flavonoids, stilbenes and condensed tannins [14-15]. There are about 40 limonoids in citrus with limonin and nomilin being the principal ones [26]. Flavonoids from natural products particularly plants extract have been reported for antimicrobial and antioxidants properties [27]. The flavonoids contents of citrus plant as observed in this study have been reported to possess strong inherent ability to modify the body's reaction to microorganism [14]. this will be a very useful property in the microbial decontamination of surface water. Quercetin, myricitin, rutin, tangeritin, naringin and hesperidin are found amongst the common flavonoids in citrus fruits [15].

The leaves, fruits, stems and roots of citrus sp like *C. aurantifolia*, *C. aurantium*, *C. paradise Macfed* and *C. Sinensis* are employed in the treatment of malaria and fever in herbal medicine [28]. The medicinal properties of these phytochemicals present in the citrus plant will be an added advantage to the quality of water when these plants are used for purification, as it will confer medicinal properties to the purified water.

The present study also found that anthraquinone and saponine was completely absent in *Citrus sinensis* leaf, stem, seed and bark. Javed *et al.*, [29], however reported the presence of terpenoids, flavonoids, alkaloids, glycosides, saponins and steroids in the peel of *Citrus reticulata* fruit. This discrepancy could be associated with the findings of earlier researcher who reported that not all phytochemicals are present in all plant and that those present differ with the organs or parts of the plant [30].

Bacterial species identified in the river, stream and pond water samples (untreated) were mostly members of *Enterobacteriaceae* family. These bacterial species include *Salmonella*, *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Enterobacter*, *Citrobacter freundii*. These bacterial isolates were found to be similar to those that are commonly observed in contaminated water bodies and other aquatic environments. Prasai *et al.*, [31], also identified *Escherichia coli*, *Enterobacter* spp, *Citrobacter* spp, *Pseudomonas aeruginosa*, *Klebsiella* spp, *Shigella* spp, *Salmonella typhi*, *Proteus vulgaris*, *Serratia* spp, and *Vibrio cholera* in water samples from Kathmandu valley, Nepal

The mean viable counts of pond water samples recorded in this study were generally higher than the set standard for water meant for drinking purposes by WHO, [32], and EU, [33]. The presence of these bacterial species in the water samples possibly suggest that the pond water in the study area have been contaminated with wastes either of human or animal origin. This means that using this water without treatment could be hazardous to human and animal health. This observation is line with the studies conducted on surface water in Malawi by Pritchard *et al.* [34], in Bamenda, Cameroun by Yongabi *et al.* [35], in Abeokuta, Ogun State, Nigeria by Ojekunle *et al.* [36] and in Guma Local Government Area, Benue State, Nigeria by Ichor *et al.* [37]

Drinking water contaminated with these organisms can cause stomach and intestinal illness including diarrhea and nausea, and even lead to death. These effects may be more severe and possibly life threatening for babies, children, the elderly or people with immune deficiencies or other illnesses [34]

Citrus limon also demonstrated significant activity, the leaf and bark material of *Citrus limon* at all concentrations tested (0.1, 0.2, 0.3, 0.4 and 0.5 g/l) completely eliminated the bacteria from the stream water after 24 hours of treatment. However, the stem and seed only show complete elimination at concentrations of 0.4 and 0.5 g/l (Table 2). *Citrus sinensis* also produced considerable decontamination of the microbial load from the stream water, However, the stem and seed material significantly ($p < 0.05$) reduced the amount of the bacteria count but could not attend complete elimination, even at the highest doses (0.5 g/l) (Table 3). This finding reflect increased concentration of antimicrobial agent as the treatment dose increased, this is in line with the findings of Yusuf et al., [38], Tsado *et al.*, [39], Ibrahim et al., [40], who reported that increased in concentration of plant material resulted in corresponding decreased in bacterial load. This finding is in line with the previous studies that reported that *Citrus sinensis*, *Citrus aurantifolia* and *Citrus limon* showed a very good antimicrobial activity against *E. coli*, *S. typhi*, *B. subtilis*, *K. pneumoniae* and *S. aureus* [41]. This antibacterial activity may be indicative of the presence of metabolic toxins or broad spectrum antibiotic compounds Amandeep *et al* [41]. Ekwenye and Edeha [42], reported antibacterial activity of *Citrus sinensis* leaf against the test organisms taken in their study (*E. coli*, *K. pneumoniae*, *S. aureus*).

MIC is the lowest concentration of antibacterial substance required to produce a sterile culture [20]. It is worthy of note that MBC values obtained for the plant materials against the pathogens are higher than MIC, indicating that the plant materials are bacteriostatics at lower concentrations and bactericidal at higher concentrations [43]. This suggests that these plant materials, when used traditionally as antimicrobials inhibit bacteria growth without necessarily killing the bacteria and since most of the traditional preparations lack specific concentrations, this may thus account for the use of large quantity of the extracts by traditional medical practitioners for the treatment of their patients. In this study, the growth of *P. aeruginosa*, *S. aureus*, and *E. coli*, which are already known to be multi-resistant to different antibiotics, was inhibited by the citrus plants species, thus indicating the antimicrobial activities of the plants against diverse organism.

5. Conclusion

It is concluded that the leaf, stem bark and seed of citrus species contains various phytochemicals and have the potentials to serve as a disinfectant for water treatment. The need to exploit the potential of these citrus plants may offer cheap, and environment friendly methods of tackling water contamination and may possibly overcome the hazards of using synthetic compounds.

Compliance with ethical standards

Acknowledgments

The authors would like to appreciate the Tertiary Educational Trust Fund of Nigerian and Federal University of Technology Minna for the research grant (TETFUND/FUTMINNA)/2017/05).

Disclosure of conflict of interest

The authors declared no conflict of interest exist.

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How to cite this article

Okunlola BM, Ijah UJJ, Yisa J and Abioye OP. (2019). Phytochemical and phyto-disinfectant properties of citrus species (*Citrus limon*, *Citrus aurantifolia* and *Citrus sinensis*) for pond water purification. *GSC Biological and Pharmaceutical Sciences*, 8(2), 34-44.
