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Antimicrobial Activities of *Cymbopogon citratus* and *Ximenia Americana* Leaf Extracts Against Some Selected Bacterial and Yeast Clinical Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Increasing emergence of resistance to antibiotics by pathogenic microorganisms worldwide necessitates the need for finding new antimicrobial agents with minimal resistance and side effects. This study was carried out to investigate the phytochemical content and antimicrobial activities of two ethno-medicinal plants namely: *Cymbopogon citratus* and *Ximenia Americana*. Methanol and aqueous were used as solvent for a soxhlet and aqueous percolation extraction techniques to obtain the crude extracts of the named plant parts. Tannins, steroids, reducing sugars, tritapenoids and Flavonoids were found present in these plant extracts. GC-MS analysis done in this study indicates the presence of some basic phenolic compounds, such as; Cyclohexane-1-3,5-trione & 2-phenyl-1,4-benzopyrone, in the *C. citratus* extract and methyl guanidine & 3-meyhylheptyl acetate in the *X. americana* extract, which have been attributed with numerous antimicrobial effects onmicrobial pathogens. Using an agar well diffusion bioassay technique the *C. citratus* extracts shows; both the extracts are active against *E. coli* and *P. aeroginosa*. While *X. americana* extracts shows a higher activity against *C. albican*. However the MIC/MBC/MFC of all the extracts shows that known of the extracts has an active viability below 12.5µg/ml.

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1. INTRODUCTION

Plants naturally contain phytochemical substances that produce definite physiological action in human body, especially nontoxic plants [1]. A good example is *C. citratus* (lemongrass), which belongs to the Gramineae family [2]. Its leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5cm [3]. Another is *X. americana* (yellow plum) a semi-scandent shrub with small elliptic leaves and whitish to yellowish-green flowers borne in small cymes and are found throughout tropical and subtropical countries in Africa, India, Central and Southern America. *C. citratus* and *X. Americana* are traditionally used in the treatment of ailments like diarrhea, dysentery, fever tuberculosis, malaria and hypertension etc. [4].

With increasing number of bacterial strains resistant to various antibiotics, many attempts to use the antimicrobial potential of plants have been done. On the other hand emergence of resistant strains among different pathogenic organism such as *E. coli, P. aerogenosa, S. aureus* and *C. albicans,* are causing problems in treating different diseases [5]. Among other examples, is a bacterial strain known as *S. aureus.* Which is a gram-positive coccus bacterium; its cells form grape-like clusters when viewed under an appropriate microscope. *S. aureus* is one of the main causes of nosocomial and community-acquired infections [1].

P. aeroginosa is an aerobic gram-negative bacillus considered to be an opportunistic pathogen [6]. It is a highly versatile and facultative anaerobic bacterium, which can survive harsh and unstable conditions [7]. *P. aeroginosa* infections are difficult to eradicate because of their elevated intrinsic resistance as well as their capacity to acquire resistance to different antibiotics [8]. *C. albicans* represents one of the yeast species of special importance to human health [9]. This can cause a wide variety of infections and aberrations. These include oropharyngeal and vulvovaginal candidiasis [10]. *C. citratus* is used as traditional folk medicine in the treatment of nervous, gastrointestinal disturbances fevers and hypertension [11]. As a medicinal plant, lemon grass has been considered a carminative and insect repellant. Therefore, the need to carry out research on the phytochemical content and actual antimicrobial activity of this herb is not actually a luxury but a necessity.

2. MATERIALS AND METHODS

2.1 Collection and Handling of Plant Materials

The leaves of the two plants: *X. Americana* and *C. citratus* were collected, from Jibga town, of Bebeji L. G. A. and the Botanical Garden of the Department of Plant Science, Bayero University, Kano-Nigeria, respectively. Identification was authenticated by staff of the herbarium of the Department of Plant Science of the same University, using standard reference guides. The leaves were shade dried and ground to powder using mortar and pestle as describe by [12] and stored in air dried containers until required for further use.

2.2 Extraction of the Plant Materials

The plant materials were extracted using aqueous and organic (methanol) solvents in accordance with the protocol of [13]. For aqueous extraction, 50g of the powder was weighed using bench top electric balance and percolated with 500ml of sterile distilled water. For ethanol and methanol extraction, 50g of the powdered leaves was extracted using soxhlet extractor. Crude extracts of all the set-ups was then obtained from the individual systems using water bath aspiration technique. 1g/ml stock solution of the individual set-ups was then prepared using DMSO as the final solvent in screw capped *bijou* bottles.

2.3 Phytochemical Screening of Extracts

Extracts obtained above were analyzed for the presence of saponins, amino acids, flavonoids, reducing sugars, tannins, steroids, tritapenoids and others.

2.4 Test for Saponins and Reducing Sugar

Saponins and reducing sugar were detected following the procedure of [14]. A persistent froth indicates a positive test. So also for reducing sugar 1ml of each fraction in separate test tubes, 2.0ml of distilled water was added followed by addition of fehling's solution (A+B) and the

mixtures were warmed. Appearance of brick red precipitate indicates the presences of reducing sugar [14].

2.5 Test for Amino Acids

This was carried out according to the method described by [5]. One ml of the extract was treated with few drops of ninhydrin reagent. Appearance of purple colour showed to presence of amino acids.

2.6 Test for Flavonoids

To 3cm^3 of the extract was added 1cm^3 of NaOH, a yellow colouration indicates a positive test of flavonoids [15].

2.7 Test for Reducing Sugars

To 1ml of each fraction in separate test tubes, 2.0ml of distilled water was added followed by addition of fehling's solution (A+B) and the mixtures were warmed. Appearance of brick red precipitate at the bottom of the test tube indicates the presences of reducing sugar in accordance with [16].

2.8 Test for Tannins

This was carried out according to the method described by [15]. To 5cm^3 of the extract, a few drops of 1% lead acetate were added. Formation of a yellow precipitate indicated the presence of tannins.

2.9 Test for Steroids

Two milliliters of the extracts were evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet colour in the supernatant layer denotes the presence of steroids [16].

2.10 Test foe Triterpenoids

This was carried out according to the method described by [17]. Ten mg of the extract was dissolve in 1ml of chloroform, 1ml of acetic anhydride was added following the addition 2ml of conc. H_2SO_4. Formation of reddish violet colour indicated the presence of triterpenoids.

However other qualitative and quantitative phytochemical screening was done to both the plant part extracts using standard protocols by [18].

2.11 Preparation of Test Extract Concentration

Four varying extract concentrations (100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml) were prepared from the stock solution (1g/ml) using serial doubling dilution method as described by [16].

2.12 Collection of Test Isolates

Stool and excretory tract isolates were collected from the microbiology laboratory of Aminu Kano teaching hospital (AKTH), Bayero University, Kano, Nigeria. The isolates were further analyzed and confirmed using biochemical and completed tests as described by [5]. Maintained on slants of nutrient agar and potato dextrose agar refrigerated (4^0c) until required for use.

2.13 Bioassay Procedure

Inoculums' Standardization: A loop full of the test isolates was picked using a sterile wire loop and emulsified in 3-4mls of sterile physiological saline followed by proper shaking. The turbidity of the suspension was matched with that of 0.5 McFarland standards. Thus: producing standardized inoculums [19].

Sensitivity Testing of the Extracts: Standardized inoculums of each isolate were swabbed onto the surface of nutrient agar in separate petri dishes. Wells are made on the plates using sterile Cork-borer and extracts (0.1ml) each. The plates were then allowed to stand for 30minutes of the extracts to diffused into the agar, after which the plates were incubated aerobically un-inverted at 35-37^{0}c for 24hours [5]. This was followed by measurement of zone of inhibition formed by the test organisms around each of the extract and standard antibiotic [16].

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts: A serial doubling dilution using distilled water to obtain four different concentrations and nutrient broth i.e. 2ml each were dispensed into sterilized test tubes. Specifically 0.1ml of standardized inoculums (3.3 x 10^6cfu/ml) was added to each of the test tubes above.

Tubes containing broth and plant extract without inocula served as positive control while tubes containing broth and inocula served as negative control. The tubes were incubated aerobically at 35^0C for 24 hours and observed for the least concentration without turbidity [19].

Determination of the Minimum Bactericidal Concentration (MBC) of the Extracts: Nutrient agar plates were separately inoculated using culture tubes that show no turbidity (MIC) and the plates were inoculated at 35^oC for 24hours. The highest dilution that yielded no growth was recorded MBC [16].

3. RESULTS

3.1 Physical Properties and Extraction Yields

Result obtained for the extraction indicated that *X. americana* gave the highest yield of aqueous extract of 16.8 %, while highest organic solvent yield of 24.8% was obtained from the methanol extraction of the same *X. Americana* (Table 1).

Table 1. Physical properties and extraction yields for the two plants evaluated

Plant	Leaf Extract	Weight (g)	Colour	Texture	Yield (%)
C. citratus	Methanol	5.3	Dark green	Gummy	10.6
	Aqueous	2.8	Dark brown	Crystalline	5.60
X. americana	Ethanol	8.4	Dark green	Gummy/oily	24.8
	Aqueous	12.4	Dark green	Gummy	16.8

Table 2. Qualitative and quantitative phytochemical content of both the organic and aqueous extracts *C. citratus* Plant Parts "values are listed as mean/average +/- mean/standard deviation", accordingly

S/N	Phytochemical	Methanolic Extract (mg/g dry/wt)		Aqueous Extract (mg/g dry/wt)	
1	Flavonoid	+	7.130 ± 2.452	+	5.410- ± 1.206
2	Alkaloid	+	5.553 ± 0.957	+	7.366 ± 0.513
3	Saponins	+	1.684 ± 0.220	+	0.381 ± 0.001
4	Phytosterols	+		+	
5	Phenols	+	16.947 ± 1.020	+	12.806 ± 1.103
6	Terpenoids	+	3.540 ± 0.151	+	1.510 ± 0.251
8	Triterpenoids	+		+	
9	Tannins	+	9.510 ± 3.836	+	7.020 ± 1.278
10	Cardiac glycoside	+	2.540 ± 0.151	+	1.170 ± 0.238
11	Anthraquinones	+	0.095 ± 0.102	_	0.180 ± 0.033
12	Anthocyanins	_		+	
13	Phlobatannins	+		+	
14	Flavonols/flavones	_		+	
15	Coumarins	_		+	
16	Quinones	_	2.140 ± 0.110	+	1.112 ± 0.143
17	Resins	+		_	
18	Amino acids	+		+	
19	Chalcones	+		_	
20	Vitamin A	_		_	
21	Vitamin D	+		_	
22	Acidic compound	+		_	

Key: + = Presence - = Absence

3.2 Results for Phytochemical Screening

Phytochemical analysis of the extracts (Tables 2 & 3) revealed the presence of tannins, saponin, alkaloids, flavonoids, amino acids, phenols, triterpenoids, terpenoids, steroids and phlobatanins. GC-MS analysis done indicates the presence of some basic phenolic compounds, such as; Cyclohexane-1-3,5-trione & 2-phenyl-1,4-benzopyrone (Fig. 1) and methyl guanidine & 3-meyhylheptyl acetate (Fig. 2) with the basic biochemical structures (table 4). Tannins, steroids and flavonoid in all the plants extract and was common to both organic and aqueous extracts tested (Table 2 & 3 below). Vitamin A were however found lacking in all the extracts tested.

3.3 Results for the Bioassays

Antimicrobial activity of the extracts indicated that all the extracts were active against the bacterial and yeast isolates tested. Methanol extract however demonstrated a higher activity against the organisms tested than aqueous extract. *P. aeroginosa* is the most susceptible organisms against *C. citratus*, producing higher zone of inhibition, while *C. albicans* is the most susceptible organism, against the *X. americana* extracts. The least inhibition was observed in aqueous extracts against all the organisms. See Table 5.

4. DISCUSSION

Indeed many scientists have been trying to developed standard capable therapeutics from plant materials but lack of standard analysis and specification in findings has been always the prime retarding issue. However the plants extracts used in the researched were normally used as tradition remedies, so it's logical for these extracts to be active on some human pathogens, however specification, purifying and formulation are the main aims of this researched. Water is normally used as solvent cause of it universal nature, but DMSO was also use in the cause of this work. It was used as a solvent in preparing the stock solution, because the methanol and ethanol extracts did not completely dissolve in water, as initially used, even when used in fractions, while DMSO was able to dissolve all the extracts and does not have any effect on the test isolates as tested and

Table 3. Qualitative and quantitative phytochemical content of both the organic and aqueous extracts *X. americana* Plant Parts "values are listed as mean/average +/- mean/standard deviation", accordingly

S/N	Phytochemical	Methanolic Extract (mg/g dry/wt)		Aqueous Extract (mg/g dry/wt)	
1	Flavonoid	+	5.130 ± 2.452	+	6.410- ± 1.206
2	Alkaloid	+	6.553 ± 0.957	+	9.281 ± 0.413
3	Saponins	+	0.484 ± 0.220	+	0.221 ± 0.041
4	Phytosterols	+		+	
5	Phenols	+	18.947 ± 1.020	+	14.806 ± 1.133
6	Terpenoids	+	2.510 ± 0.221	+	1.640 ± 0.210
8	Triterpenoids	+		+	
9	Tannins	+	7.310 ± 3.436	+	5.020 ± 1.278
10	Cardiac glycoside	+	2.211 ± 0.050	+	1.120 ± 0.230
11	Anthraquinones	+	1.095 ± 0.102	_	1.081 ± 0.033
12	Anthocyanins	_		+	
13	Phlobatannins	+		+	
14	Flavonols/flavones	_		+	
15	Coumarins	_		+	
16	Quinones	_	6.830 ± 0.101	+	3.350 ± 0.140
17	Resins	+		_	
18	Amino acids	+		+	
19	Chalcones	+		_	
20	Vitamin A	_		_	
21	Vitamin D	+		_	
22	Acidic compound	+		_	

Key: + = Presence - = Absence

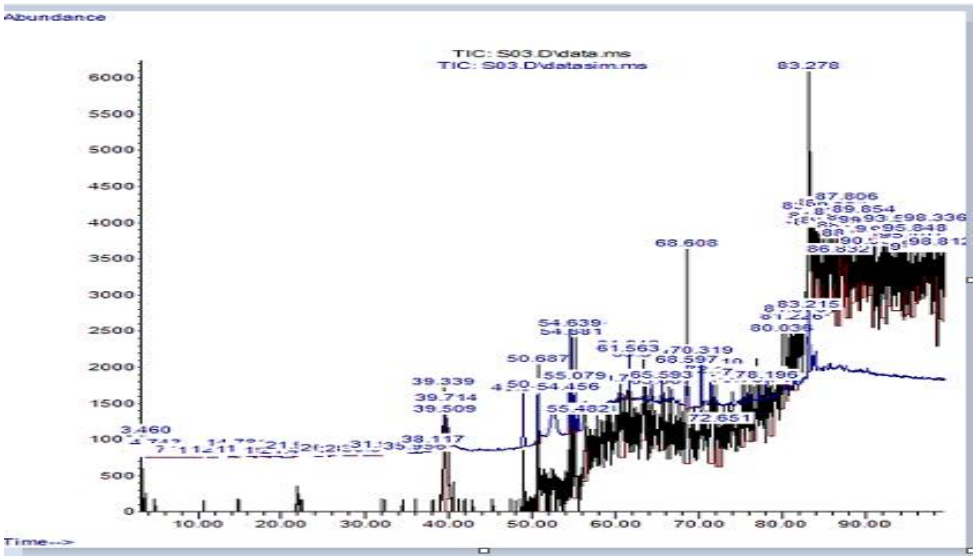


Fig. 1. GC-MS spectrum of methanolic leaf extract of *C. citratus*

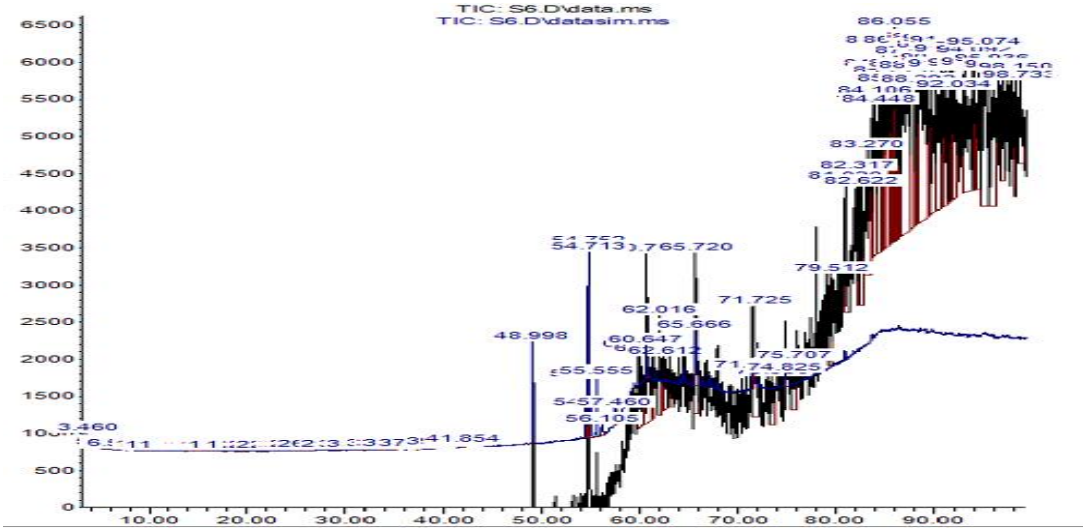


Fig. 2. GC-MS spectrum of methanolic leaf extract of *X. Americana*

used as control. Tannins, steroids, reducing sugars, tritapenoids and Flavonoids were found present in these plant extracts. Using an agar well diffusion bioassay technique the *C. citratus* extracts shows; both the extracts are moderately active against *E. coli* and *P. aeroginosa*. While *X. americana* extracts shows a higher activity against *C. albican* as seen in table 5. However the MIC/MBC/MFC of all the extracts shows that none of the extracts has an active viability below 12.5µg/ml (table 6). GC-MS analysis done in this study indicates the presence of some basic phenolic compounds, such as; Cyclohexane-1-3,5-trione & 2-phenyl-1,4-benzopyrone, in the *C. citratus* extract (Fig. 1) and methyl guanidine & 3-meyhylheptyl acetate in the *X. americana* extract (Fig. 2), which have been attributed with numerous antimicrobial effects on microbial pathogens. Octanoic acid belong to a class of medium chain saturated fatty acids, it has antibacterial, anti-viral and anti-fungal properties and it can help to treat health problems associated with the over growth of yeast , such as a virginal yeast infection, candida and thrush.

Gentamycin (antibiotic) was used as a control and it demonstrate a higher activity against the

test isolates than all the test extracts. This might be due to the fact that, this agent is in its pure state and has undergone a series of refining process that have established it as standard. Another reason might be due to the fact that these extracts were in crude form and hasn't undergone further refining and purification. The antimicrobial activity seen from this plants extract could be attributed to the presence of phenols, flavonoid and terpenoids. The presence of flavonoid, phenolic acid and tannin could contributes to numerous antibacterial/fungal properties, together with also the presence of alkaloid and other phytometabolites. These results are compared with the work reports of: [20 & 21] on "*C. citratus* invitro antibacterial activity" and [22] on *X. americana* leave extracts pharmacology.

Table 4. Probable peaks obtained from GC-MS analysis of *C. citratus* and *X. Americana* leaf extracts

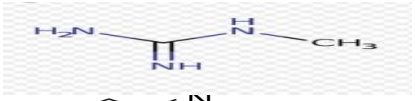
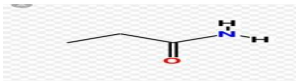
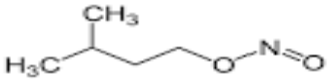
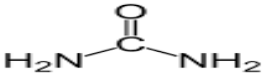
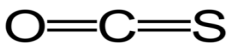
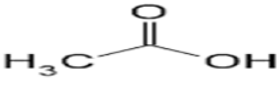
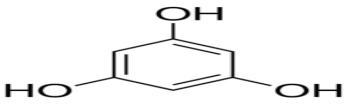
S/N	RT	Area	Name of compounds	Structure
1.	92.123	2.49	Guanidine methyl	
2.	68.602	5.14	Aminoacetonitrile	
3.	93.582	3.64	Propanamide	
4.	39.714	3.4	Amyl nitrate	
5.	39.339	3.28	Urea	
6.	83.729s	6.93	Pentanoic acid, 4-methyl	
7.	60.779	2.95	Carbonyl sulfide	
8.	71.51	3.26	Methanamine, N-hydroxy-N-methyl N	
9.	55.116	2.51	Acetic acid	
10.	75.211	1.62	Cyclohexane-1,3,5-trione	

Table 5. Mean Results for the Bioassay of *C. citratus* and *X. americana* Leaf Extracts in (mm)

Extracts Source	Test Organisms	Methanol Extracts (µg)				Aqueous Extracts (µg)				SG (30µg)
		12.5	25	50	100	12.5	25	50	100	
	E. coli	12.5±0.071	14.5±0.352	20±1.012	230.523±	8.5±0.222	8.5±1.011	15±1.238	18.5±0.7001	32±1.190
	S. aureus	9.5±1.118	10.5±1.320	11.5±1.721	14.5±0.816	7.5±0.165	14.5±0.643	16±1.621	18.5±0.932	22±0.001
C. citratus	*P. aeroginosa*	19±2.001	20.5±1.912	21.5±0.021	23.5±1.065	23±0.551	17±1.028	17.5±1.112	20±1.090	30±0.004
	C. albican	7.5±1.099	8.5±0.995	12.5±1.467	15±0.832	7±1.382	9±1.001	12±0.772	13±1.765	18±0.054
	E. coli	17±1.035	21±1.742	23±0.118	25±1.563	16±2.001	20±1.234	20±1.423	23±0.003	32±1.642
X. americana	*S. aureus*	19±0.085	21±1123	23±1.006	24±1.654	18±1.009	23±0.011	24±1.453	25±0.743	32±0.165
	P. aeroginosa	22±0.011	27±0.687	26±1.222	29±1.907	21±0.743	25±0.674	26±0.436	28±0.088	29±0.042
	C. albican	20±1.043	27±1.243	28±0.541	30±1.005	20±1.250	24±0.642	24±0.423	25±1.391	180.701

Key:SG: Standard Gentamycin (30µg)

Table 6. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/fungicidal Concentration (MBC) of *C. citratus* and *X. Americana* Leaf Extracts

Extracts Source	Test Organism	Methanol Extract (µg/ml)		Aqueous Extract (µg/ml)	
			MIC	MIC	MBC
C. citratus	*E.coli*	12.5	*	*	*
	S. aureus	*	*	*	*
	P. aeroginosa	12.5	12.5	12.5	*
	C. albicans	*	*	*	*
X. americana	*E.coli*	*	*	*	*
	S. aureus	*	*	*	*
	P. aeruginosa	*	*	*	*
	C. albicans	12.5	*	*	*

The MIC and MBC of all the extracts shows that known of the extracts MIC/MBC goes above 12.5µg/ml.
*Key: * = MBC or MIC is greater than 12.5.*

5. CONCLUSION

Scientific researches shouldn't be concluded as confirmed, without repetition and specification, but so far for the two plant extracts used in this researched, similar actualization has been done by other scientists and confirmed the biological activity of their extracts. So with this work, we can conclude that *C. citratus* and *X. americana* extracts are potentially active against some specific pathogens as shown in this work.

6. RECOMMENDATION

Further research should be carried out to qualitatively determine the actual active biometabolites (phytochemicals) of these plants, the spectrum wideness of its extracts and its pharmacodynamics/pharmacokinetic.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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