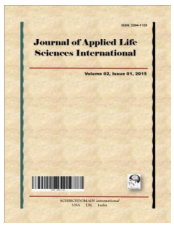

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Preliminary Phytochemical and Antimicrobial Activity of *Citrus x limon* (L) Burm. f. (lemon) Leaf Extract against Some Pathogenic Microorganisms

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Authors' contributions

This work was carried out in collaboration between all authors. Author JUE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SAG, GM and SYD handled the critical revision of the manuscript. Authors JUE, MBB and DAI monitored the experimental procedures. Authors JUE and JHD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

To authenticate the claims by local users of *Citrus x limon* (lemon) in the control of some human infections, preliminary phytochemical screening and antimicrobial evaluation of lemon leaf extract was investigated Extraction of plant leaves was performed using successive reflux, with n-hexane, ethyl acetate, ethanol and water as extracting solvents. Ethanol gave the highest percentage yield of 10.93% followed closely by ethyl acetate with 9.42% while water extract gave the lowest yield of 3.67%. 11 phytochemicals classes were present in the leaf extract and they include alkaloids, flavonoids, saponins, anthraquinon, cardiac glycosides, tannins, steroids, terpenes, resins, phenols

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and volatile oils. Saponins was absent in the n-hexane extract and ethyl acetate extracts. Agar diffusion and tube dilution methods were used to evaluate the antimicrobial activities. The test organisms were susceptible to the ethyl acetate and ethanol extracts but resistant to the oil and aqueous extracts. *Salmonella paratyphi* C was more susceptible to the extract; ethanol extract precisely having the highest mean zones of inhibition (MZI) of 13.00±1.00 mm followed closely by *Salmonella paratyphi* B with 12.33±2.89 mm while *Salmonella paratyphi* A was least susceptible to the plant extract having MZI of 6.33±5.51 mm. The activity of the plant extract generally as shown by the MZI is within the intermediate and resistant zones when compared with the control. Results of the acute oral toxicity shows no adverse toxic effect on the animals and the LD_{50} is above 5000 mg/kgbw. Based on the result of these research, *Citrus limon* leaf extract may be considered safe for consumption and may be use to control infections by the test organisms in high dosage.

Keywords: Citrus limon; successive; extraction; phytochemical screening; anti-microbial activity; LD_{50}; acute toxicity.

1. INTRODUCTION

The beginning of modern medicines dates from the 19th century, with the development of the germ theory of disease; the use of antiseptics and anaesthesia in surgery, a revival of public health measures and better sanitation. Modern medicine in the 20th century has been characterized by the introduction in the United Kingdom of the National Health Service [1]. Advances have also been notable in the treatment of mental illness through both psychotherapy and the administration of drugs [1]. Plant based medicines are important therapeutic weapons to cure human diseases [2].

Nature is seen as source of medicinal agents for thousands of years, since the beginning of man on earth. In Nigeria, almost all plants are medicinal and the application of medicinal plants especially in traditional medical practice is currently well acknowledged and established as a viable profession [3].

Plant-based medicines have been used for decades especially in rural areas to prevent or even eliminate diseases worldwide and have proven to be promising in their actions [3]. Herbal medicines otherwise called herbal drugs are generally of natural plant parts such as stem, leaves, roots, flowers, stem bark, seeds, bulb [4]. In addition to providing the animal kingdom it's food, fuel and shelter, plants accumulate other phytochemical constituents - the secondary metabolites which are produced as by-products and are sometimes not directly useful to them. These secondary metabolites give plants their medicinal value. Some of these include alkaloids, tannins, saponins, flavonoids, antraquinones, glycosides, terpenines, essential oils, resins [4]. Therapeutic medicines of plant origins are marketed aggressively across the world with good results and better knowledge, so it is a time to take a look at the advantages of plants as sources of therapy over modern therapy or the combination of both. This can be achieved by scientific research in the field of Plant-based therapy [5]. The advantages of using herbal medicines is the fact that they are relatively safer and less costly when compare to the synthetic drugs [6]. In addition, herbal medicine is a complex combinations of different phytochemical components possessing different mechanisms of actions on microbial cells, which makes it difficult for pathogenic organisms to develop resistance to their use [7]. The local uses of the leaf of this plant under study for the treatment respiratory disorder and suppression of typhoid fever in some part of Nigeria, have necessitated the scientific study of its potency against the causative agents of these infections.

2. MATERIALS

2.1 Plant

Leaves of *Citrus limon* (lemon) were used as the sample under investigation, plant leaf were collected from house-hold gardens in Bosso estate, Bosso Minna Niger State, Nigeria during the dry season between August and September. The plant was identified by the local herbal maketers in Gwari Market, Niger State Nigeria and Authenticated by Mr. John Atogwe of the herbarium department of National Institute of Pharmaceutical Research and development (NIPRD), Idu Abuja. Nigeria. The leaves were air-dried under shade for three weeks until a constant weight was obtained, furthermore, they were milled with a milling marchine (Labworld NAVBHART, with serial No. R66902 by MOTOR MFG, CO. Mumbai-India), the pulverized plant samples were sieved with a 150 µm pore size

filter to obtain a fine powdered-like texture. This was done to enhance the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active principles. The pulverised plant samples were then stored in amber bottles and kept in a cool and dried environment under room temperature until it is required for usage.

Fig. 1. Leaves of *Citrus limon*

2.1.1 Test organisms

The test microorganisms are species of *Salmonella enterica serotype paratyphi* A, *Salmonella enterica serotype paratyphi B, Salmonella enterica serotype paratyphi C, Salomonella enterica serotype* Typhi*, Klebsiella pneumoniae, Streptococcus pneumoniae and Streptococcus pyogenes*. The organisms were collected from the stock cultures in the Department of Microbiology, Federal University of Technology Minna, Niger State. Nigeria. All the test organisms' identity were confirmed using standard laboratory methods according to [8].

3. METHODS

3.1 Extraction

The reflux extraction method was used to obtain the crude extract at a temperature of 30℃ for 2 hours and the extraction was carried out successively (i.e. beginning with the non-polar solvent to a polar solvent); specifically n-hexane (polarity index= 0.0_P') for the extraction of low polar constituents (e.g. volatile oil, hydrocarbons, fatty acids, sterols), ethyl acetate (polarity index= 4.4_P'), ethanol (polarity index = 5.2_P') and water (polarity index = 10.2_P'). This was achieved by dissolving 100 grams of the dried plant material in 400 ml of the extracting solvent n-hexane (1:4). After 2 hours of refluxing, the mixture was filtered through whattman No 1 filter paper with pore size of 0.7 µm to obtain a clear filtrate which was concentrated to a semisolid substance with the use of rotary RE-6000 rotary evaporator at 50℃. The concentrate was then freeze dried using the LGJ-10 lyophilized at a temperature of -30℃ to a fine powder which was stored in an air-tight amber bottle and kept in the refrigerator for further analysis while the marc was air-dried for 1 hour and was extracted with the next solvent in increasing order of polarity. This procedure was repeated using the other solvents until the dried marc (residual plant materials after filteration) was finally extracted with water (by decoction method, which involve mixing the plant materials in water and heating to boil for 2 hours without refluxing).

The percentage yield of crude extracts were determined using the equation below.

> Percentage yield = [(weight of extracts/ Weight of dry plant material) x 100/1]

3.2 Phytochemical Analysis

The phytochemical analysis of the plant extract was done according to the method of [9,10]. 12 different phytochemicals were analysed for, these includes steroids, cardiac glycosides, phenol, flavonoids, saponins, tannins, terpenoids, and glycosides.

3.2.1 Test for phenols

One milligram (1 mg) of crude extract and Iron (III) chloride were mixed and vortexed for 2 minutes. The Formation of a deep bluish green colouration is indicative of the presence of phenols.

3.2.2 Test for flavonoids

Five millilitre (5 ml) of dilute ammonia was added to the aqueous portion of the extract (1 mg), followed by concentrated sulphuric acid (1 ml). A yellow coloration that disappears on standing indicates the presence of flavonoids.

3.2.3 Test for saponins

Two grams (2 g) of the powdered sample was heated to boil in 20 ml of distilled water and then filtered. 10 ml of the filtrate was next vortexed with 5 ml of distilled water and shaken vigorously for the generation of persistent froth. The frothing was mixed with 3 drops of olive oil and re-shaken again vigorously, it was then observed for the formation of emulsion.

3.2.4 Test for tannins

Five (0.5 g) of the dried plant samples was heated to boil in 20 ml of distilled water in a test

tube and the mixture was filtered. A few drops of 0.1% ferric chloride was added dropwise and it was observed for brownish green or a blue-black coloration, indicative of tannins.

3.2.5 Test for terpenoids (Salkowski test)

Five (5 mg) of each extract was mixed in 2 ml of chloroform, and about 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

3.2.6 Test for steroids

To 2 ml of acetic anhydride was added to 0.5 g of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

3.2.7 Test for cardiac glycosides (Keller-Killani test)

Five (5 ml) of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution and was further treated with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer indicative of the presence of cardiac glycosides according to [11].

3.2.8 Test for anthraquinone

Borntrager's test – To show the presence of free Anthraquinone, 0.5 g of the powdered leaf samples were taken in dry test tubes. Ten millilitres of Chloroform were added to each and the mixtures was shaken for 5 minutes. It was next filtered and an equal volume of ammonia solution added to the filtrate and thoroughly shaken. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones.

3.2.9 Test for volatile oils

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. The plant materials were distilled with water by steam distillation and the distillates were collected in a graduated tube. The aqueous portion which separates automatically was returned to the distillation flask. The formation of emulsion which floats on top of the aqueous phase owing to its low density is indicative of the presence of volatile oils.

3.2.10 Test for resins

Solutions of 5ml n-hexane were made using 0.1 g of powdered leaf samples of *Citrus limon* and was well labelled. An equal volume of copper acetate solution was added and shaken vigorously then allowed to separate. A green colour indicates the presence of resins.

3.2.11 Test for alkaloids

Ten grams (10 g) of the leaf extracts were transferred into 500 ml capacity beaker and a strong solution of ammonia was added in a quantity just sufficient to moisten the mixture and allowed stand for 10 minutes after thorough mixing. A mixture of chloroform and ethanol solution in the ratio 1:1 was added just to soak and suspend the extract. The mixtures were allowed to stand for 20minutes with occasional stirring with the aid of a glass rod stirrer. The mixtures were next filtered through a plug of cotton wool and washed twice with 2 ml Chloroform. The bulk filtrate was concentrated to dryness without overheating. The residue were cooled and dissolved in 5 ml Chloroform only. The chloroform solution was transferred to a small separating funnel and shaken with 3 ml of dilute sulphuric acid. The two layers were allowed to separate, while the chloroform lower layer was drained off and discarded. Three millilitres of chloroform was further added and shaken, drained off and discarded until upper acid layer was colourless. The acid layer was made completely alkaline with strong ammonia solution; it was next tested with an indicator paper. The extracts with 3 ml of chloroform were evaporated to dryness. The residue was dissolved in 3 ml of ethanol and the following tests were carried out after neutralizing with dilute sulphuric acid. Little amounts of the ethanol solution were added in drop-wise manner to a few drops of Mayer's solution (potassium mercuric iodine) test tubes. The presence of precipitate in the above indicates the presence of alkaloids [12].

3.3 Antimicrobial Susceptibility Screening

3.3.1 Standardization of inoculum

Zero point two millilitre (0.2 ml) of 24 hours culture of each test organism was dispensed into 20 ml of sterile nutrient broth and incubated for

3 – 5 hours, this is to standardize the culture to 10^6 cfu/ml. A loopful of the standard cultures was inoculated on the media used for the antimicrobial assay [13].

3.3.2 Preparation of extract concentration

Two hundred milligram (200 mg), of the oil, ethyl acetate, ethanol and aqueous crude extract were weighed and dissolved in 5 ml each of 20% Dimethyl sulfoxide (DMSO) (20 ml DMSO was made up to 100 ml with distilled water) to give 40 mg/ml concentrations respectively.

3.3.3 Antibacterial susceptibility test of crude extract and plant oil

The antibacterial activity of the extracts were investigated using the agar-well diffusion method as described by [14] using Muller-Hinton agar, prepared according to manufacturer instructions. Wells were made in the inoculated media using sterile cork-borer (6 mm diameter) after which a little molten media was used to seal the base of the wells to prevent unwanted spread of the extracts. The media was inoculated with the standardized test organisms by the spread plate method using a sterile rod spreader to obtain uniform microbial growth. 200 µl each of the prepared extract and plant oils equivalent to the desired concentrations per millilitre was transferred into the wells with a sterile micropipette and it was well labelled, while 200 µl of 10% DMSO (free of extract and essential oil) was transferred into wells to serve as the negative control. Ciprofloxacin (5 µg/ml) was used as the positive control. This was done by transferring 200 µl of the prepared Ciprofloxacin (10 µg) into the well and the cultures with the extracts and plant oil was allowed to stand for 30 min after which it was incubated at 37°C for 24 hours. The experiment was carried out in triplicate and the mean values with the corresponding standard deviation of the inhibition zone diameters (IZD) were calculated. The performed agar well diffusion susceptibility test was based on the modified methods of the Science Laboratory Standards Institute [15].

3.4 MIC and MBC Test of Crude Extracts

3.4.1 Serial dilution and determination of mic of crude extracts

The tube dilution method was used to determine the minimum inhibitory concentration (MIC). Two fold serial dilutions of the crude plant oils and extracts were prepared to give a decrease in concentration ranging from 40, 20, 10, 5, 2.5, 1.25 & 0.625 mg/ml and respectively. This was achieved by weighing and dissolving 160 mg of either oil or crude extract (as the case may be) in a test tube labelled A (40 mg/ml) containing 4 ml of lactose broth (LB), from test tube A, 2 ml was transferred into a second test tube labelled B (20 mg/ml) containing 2ml of lactose broth. This procedure continued until a concentration of 0.625 mg/ml was obtained in the 7^{th} test tube labelled G (0.625 mg/ml) and from test tube G, 2 ml was discarded. Before every transfer from one tube to the other, each tube was well vortexed for at least 5 seconds to ensure a homogenous mixture. Control tubes of sterile lactose broth were also prepared without the addition of the plant extract but was inoculated with the test organism. All tubes were incubated in a shaker incubator at 37°C for 24 hours and after incubation, the optical density was determined using a spectrophotometer at 600 nm. The concentration/culture with the lowest optical density was recoded as the minimum inhibitory concentration.

3.4.2 Determination of MBC

The minimum bactericidal concentrations was determined using simple laboratory culture method which was achieved by sub-culturing the cultures with the lowest optical density (test tubes without turbidity) beginning with the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 - 48 hours at 37°C, after incubation, the culture concentration without visible growth on the media was regarded as the minimum bactericidal concentration.

3.5 Acute Oral Toxicity of Active Extracts

The Lorke's method of acute toxicity with slight modification was carried out on the active extracts (ethyl acetate and ethanol extracts) and this test was also intended to be used to determine the median lethal dose (LD_{50}) of the extracts using the oral gavage route of exposure [16,17]. The toxicity study was carried out using 32 Swiss albino mice (20 g to 25 g body weight) of either sex. The animals were divided into two phases; phase 1 comprises of 24 animals while phase two comprises of 8 animals. Phase 1 was further divided into two groups of 12 animals each for ethyl acetate extract and ethanol extract respectively. For ethyl acetate extract, 3 sub-

groups which comprises of 3 treated animals plus 1 control in each sub-group given a total of 4 animals per each sub-group. Each treated animal received 10, 100 and 1000 mg/kgbw respectively in triplicate while the control animal received equivalent volume (1 ml) of dimethyl sulfoxide (DMSO). In the phase 2, the two major groups comprises of 4 animals per group for ethyl acetate and ethanol extract respectively, in each group 3 animals were treated; while one served as the control receiving 2000, 3500 and 5000 mg/kgbw and the control animals received equivalent volume of DMSO (1 ml). The animals were observed for toxicity signs such as paw-licking, stretching, respiratory distress, diarrhoea and death at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and were further left for 14 days for delay mortality.

4. RESULTS AND DISCUSION

Citrus limon is a flowering plants in the rue family, Rutaceae. Research indicates its origin in Australia, New Caledonia and New Guinea [18]. Reports by local marketers and consumers have implicated it for use in the control of infections caused by both gram negative and gram positive microorganisms. The extraction result of this study reveals that ethanol is a good solvent for the extraction of citrus leaf crude extract as it gives the highest percentage yield of 10.93% followed closely by ethyl acetate extract with 9.42% while water gives the least yield of 3.67%. The result also reveals the presence of oil in the plant as hexane yielded 4.75% all from a total of 500 g of dried leaf material. It is important to note at this point that the yield obtained using a particular extracting solvent is not directly proportional to the antimicrobial activity of the extract obtained in the solvent. This because the law governing extraction is that like extracts like i.e. a polar solvent will extract a polar constituent and vice vasa. That is, the yield of the non-polar constituents obtained with a non-polar solvent might be little but might also at the same time be the active constituent in the extract. The fact that ethanol gave the highest yield could be a pointer that the highly polar constituent with polarity index as that of ethanol and very soluble in ethanol might be the most abundant in the leaf extract which is similar to the view of [19]. Phytochemical result also reveals the presence of 11 different constituents viz; alkaloids, flavonoids, saponins, anthraquinon, cardiac glycosides, tannins, steroids, terpenes, resins, phenols and volatile oils which cut across almost all solvents used for the extraction process. Though a qualitative phytochemical analysis was employed in this research which limits the its ability to ascertain the amount of each constituents but the presence of alkaloids and flavonoids buttressed the claims by [20] that Citrus green parts were consumed mainly for its abundance of volatile oils, alkaloids and flavonoids which are reported to have anticancer activities and antibacterial potential in crude extracts of different parts (viz*.,* leaves, stem, root and flower) of Lemon against clinically significant bacterial strains while flavonoids have been reported to have a large spectrum of biological activity including anticancer, antibacterial, antiviral, antifungal and antidiabetic activities [21]. Flavonoids generally are also reported to be present in glycosylated forms in plants, and the sugar moiety has been found to be an important factor in determining their bioavailability. Volatile oil was also present in the leaf extract which might be a bases for the explanation of a report on lime juice and its oil having potency for skin rejuvenation when consumed orally or applied externally. It keeps it shining, protects it from infections and reduces body odour due to presence of a large amount of vitamin-C and Flavonoids, both of which are class-1 antioxidants, antibiotic and disinfectants [21,22]. The antimicrobial activity of the plant extract may be due to the abundant presence of phytochemicals and the result of this study confirms the claims by local marketers and consumers of the use of the leaf of the plant either singly or in combination for the treatment of typhoid fever and pneumonia. Several report have also been documented on the antibacterial effect of steroids which is present in the plant under study [23]. The minimum inhibitory concentration of a particular chemotherapeutic agent is a factor that best describe the efficacy of that agent. The MIC can guide the choice of antimicrobial used in treatment by predicting efficacy. If pharmacokinetic and pharmacodynamics (PKPD) principles are observed by careful selection of a particular antimicrobial administered at an appropriate dosage, it will lead to clinical cure, eradication of carrier status of a system, and prevention of selection of resistance.

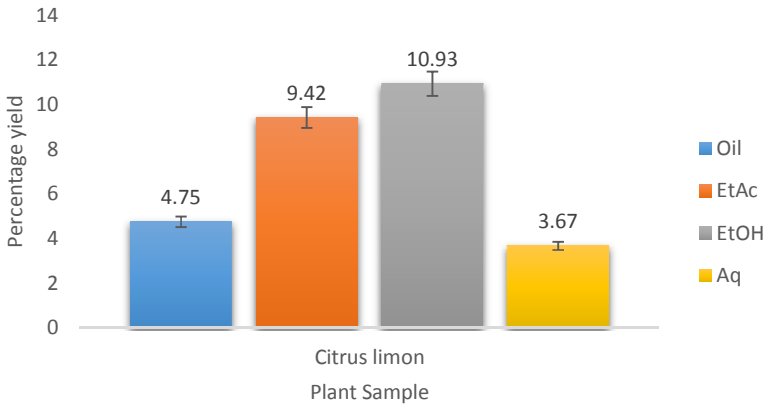

Fig. 2. Percentage yield of crude extracts (%)

Key: EtAc: ethyl acetate extracts, EtOH: ethanol extracts, Aq: aqueous extracts

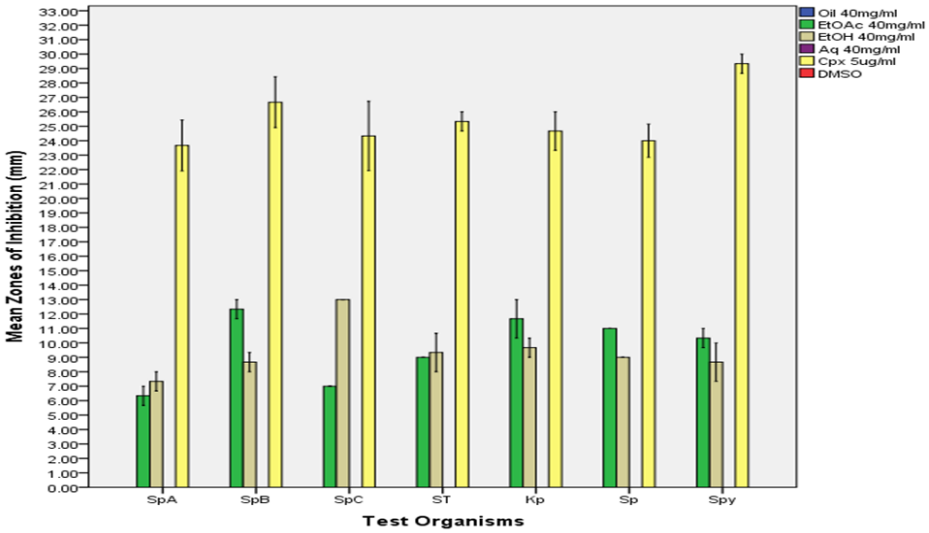

Fig. 3. Mean zones of inhibition of *Citrus limon* crude leaf extract (mm)

Key: EtAc: ethyl acetate extract, EtOH: ethanol extract, Aq: aqueous extract, Cpx: ciprofloxacin, DMSO: dimethyl sulfoxide, SpA: Salmonella enterica subsp.enterica paratyphi A, SpB: Salmonella enterica subsp.enterica paratyphi B, SpC: Salmonella enterica subsp.enterica paratyphi C, ST: Salmonella enterica subsp.enterica Typhi, Kp: Klebsiella pneumoniae, Sp: Streptococcus pneumoniae, Spy: Streptococcus pyogenes, NA: no activity

Table 1. Phytochemical properties of *Citrus limon* leaf oil and crude extracts

Plant extracts	Flavonoid	Phenols	Alkaloids	Tannins	Steroids	Cardiac gylcosides	Saponins	Terpenes	Volatile oil	Anthraquinon	Resins
CL (oil)	+	+	+	+	+	+	-	+	+	+	+
CL EtAc	+	+	+	+	+	+	-	+	+	+	+
CL EtOH	+	+	+	+	+	+	+	+	+	+	+
CL AqE	+	+	+	+	+	-	+	+	+	-	+

Key: (+): present, (-): absent

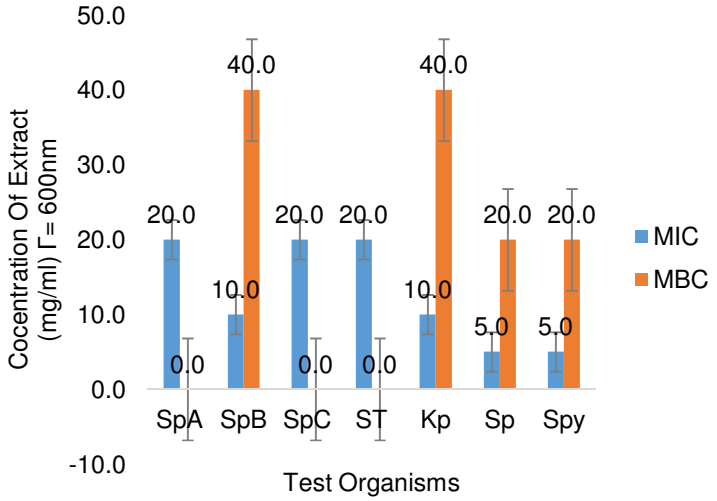

Fig. 4. Minimum inhibitory concentration and Minimum bactericidal concentration of *Citrus limon* ethyl acetate extract

Key: SpA: Salmonella paratyphi A, SpB: Salmonella paratyphi B, SpC: Salmonella paratyphi C, ST: Salmonella typhi, Kp: Klebsiella pneumoniae, Sp: Stretococcus pneumoniae, Spy: Streptococcus pyogenes

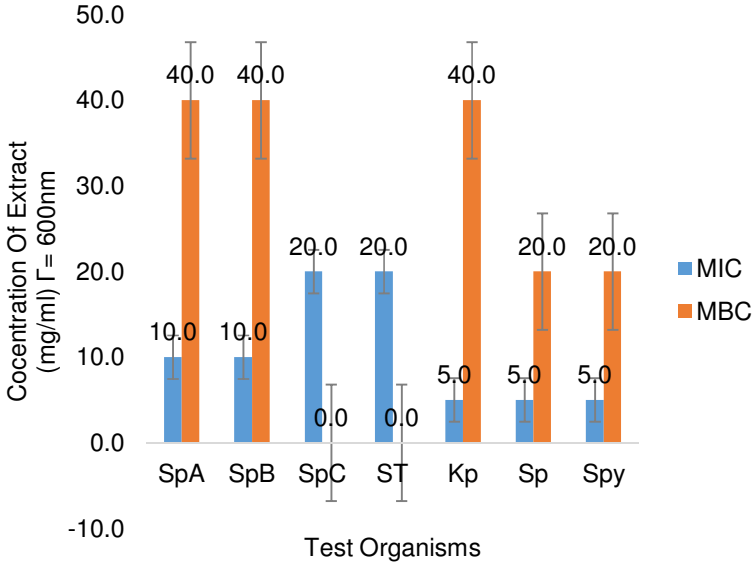

Fig. 5. Minimum inhibitory concentration and Minimum bactericidal concentration of *Citrus limon* ethanol extract

Key: SpA: Salmonella paratyphi A, SpB: Salmonella paratyphi B, SpC: Salmonella paratyphi C, ST: Salmonella typhi, Kp: Klebsiella pneumoniae, Sp: Stretococcus pneumoniae, Spy: Streptococcus pyogenes

The lowest MIC recorded in this study was 5 mg/ml, of which when the extract is purified further by subjecting the extract to other sensitive purification processes like column chromatography this concentration might drop down with increase efficacy. This has been found to be true with standard drugs like the quinolones and aminoglycosides whose efficacy depends on concentration. Whereas, some other drugs efficacy are time-dependent such as the beta-lactams [24]. Fig. 4 also shows that the MBC of the ethyl acetate extract against

Table 2. Acute toxicity result of *Citrus limon leaf* extract

		PHASE I		
Extract	**No. of Animal**	**Doses (mg/kgbw)**	**Mortality**	**Toxicity signs**
EtOAc	3	10	0	No observable sign of toxicity
	3	100	0	No observable sign of toxicity
	3	1000	0	No observable sign of toxicity
EtOH	3	10	0	No observable sign of toxicity
	3	100	0	No observable sign of toxicity
	3	1000	0	No observable sign of toxicity
DMSO	6	1ml each	0	No observable sign of toxicity
		PHASE II		
EtOAc	1	2000	0	No observable sign of toxicity
	1	3500	0	No observable sign of toxicity
	1	5000	0	Shivering, inactive after the first 2hrs, but became normal later.
EtOH	1	2000	0	No observable sign of toxicity
	1	3500	0	Inactive and loss of sensitivity At the first 30mins
	1	5000	0	Loss of sensitivity and inactive At the first 20mins.
DMSO	2	1ml each	0	No observable sign of toxicity

LD_{50} > 5000mg/kgbw for both ethyl acetate and ethanol extract

Salmonella enterica subsp. enterica serovar paratyphi A, Salmonella enterica subsp. enterica serovar paratyphi C and Salmonella enterica subsp. enterica serovar Typhi is well above 40 mg/ml for the ethyl acetate extract which was same for the ethanol extract except for *Salmonella enterica subsp. enterica serovar paratyphi* A which was exactly 40 mg/ml. In most case like this, drugs with a higher or an undetermined MBC most time is a clue to the fact that such drug might be microbiostatic in its effect against the test organism. Furthermore, the result of the MIC and MBC shows that the plant extract was more effective against gram positive bacteria than the gram negative ones. Acute toxicity result of the active extract reveals that the extract might be safe for consumption as the LD_{50} appear to be well above 5000 mg/kgbw and no death was recorded.

5. CONCLUSION

Results of the phytochemical and antimicrobial analysis of *Citrus limon* leaf extract rerveals that the leaf part of the plant contain phytochemical constituents and can best be extracted with ethyl acetate and ethanol. It also shows that only the ethyl acetate and the ethanol extracts are active against the test organisms and can therefore be use to develop drugs which can be taken in slightly higher dosage for the management and treatment of infections caused by the test organisms.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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