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Antioxidant, antimicrobial and FTIR analysis of methanol root extract of *Cnestis ferruginea* and ethanol root extract of *Citrus limon*

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Abstract

Concoctions of roots of *Cnestis ferruginea* and *Citrus limon* are used locally in the treatment of breast cancer, wounds, dysentery and tooth ache. In this study, methanol root extracts of *Cnestis ferruginea* and ethanol root extracts of *Citrus limon* were evaluated for antimicrobial, and antioxidant capacities. Preliminary phytochemical screening of the extracts was performed using standard methods. The agar well diffusion method was used to determine the antimicrobial activities of the extract against test organisms *Staphylococcus aureus*, *Bacillus subtillus, Salmonella typhi, Escherichia coli, and Candida albicans*. In addition, the minimum inhibitory concentration of extracts was evaluated using broth dilution method. The antioxidant activity was determined by total antioxidant capacity and DPPH free radical scavenging assay. FTIR analysis were conducted on a component of each extracts. Extracts from both plants showed antimicrobial activity against test organisms. The IC_{50} values were 0.069 mg/mL and 0.618 mg/mL for *Cnestis ferruginea* and *Citrus limon* respectively. Preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins and glycosides in both extracts with *Cnestic ferruginea* extract having anthraquinones in addition. FTIR analysis indicated the presence of various functional groups in both extracts that confirms the presence of the phytochemicals identified in the screening test. The results from the study showed that both extracts had antioxidant and antimicrobial properties.

Keywords: *Cnestis ferruginea, Citrus limon,* antimicrobial properties, antioxidant capacities, FTIR analysis

1. Introduction

The realm of drugs obtained from plants is vast, wider than any other source of natural products. From ancient to present day, virtually all culture and civilizations have relied fully or partially on herbal medicines due to their effectiveness, affordability, low toxicity and acceptability [1]. They have been utilized to a great degree and thus the primary health care of many developing countries relies on these herbal medicines [2-3]. Medicinal plants have attracted the attention of the pharmaceutical and scientific communities as evidence has demonstrated the promising potentials of antimicrobial, antioxidant, anti-infectious and anti-tumour plant-derived substances [4-8]. These, coupled with the fact that the unrestrained use of available antimicrobial and antioxidant regimes has led to increasing trends of resistance among emerging and re-emerging microbial pathogens [9-10]. It has been a major problem in the drug discovery industry. This has incited to a need to find new therapeutic compounds with an ideally novel modes of action [11].

Free radical oxidation in the body normally leads to several predicaments, hence causing potential diseases such as cancer, Alzheimer's disease and accelerating aging. Oxidative stress and cellular metabolism creates reactive oxygen species (ROS) that adds to the etiology, pathogenesis and progression of a few diseases including inflammations, cancer, and cardiovascular diseases[12]. Antioxidants can inactivate ROS and allow for protection from oxidative harm and are accordingly viewed as essential therapeutic and prophylactic agents against the development of diseases [13]. Some synthetic antioxidants may pose a threat to humans due to their potential side effect. In this manner, screening sources of natural products for novel antioxidant agents is a need.

Cnestis ferruginea Vahl ex DC (Connaraceae) (C. ferruginea) is a short ornamental shrub, which is sometimes a climber. It grows to about 2.5 meters high and is usually covered by dense, brown velvety hairs [14] (Hutchinson and Dalziel, 1958). The plant is widely used in West Africa for the treatment of various ailments in traditional medicine [15]. It is known to have many therapeutic uses including being a laxative, remedy for dysentery and gonorrhea [16].

The root decoction of Cnestis ferruginea (CF) Vahl DC (Connaraceae) is used in traditional African medicine in the management of psychiatric disorders [15], as an aphrodisiac, for gynaecological troubles, dysentery and urethral discharge [17]. Root extracts of *C. ferruginea* has been found to possess anxiolytic and anti-depressant effects [15], anti-stress properties [18], hepato- and nephro-protective properties [19], anti-inflamatory properties [20], increment of sexual invigoration in males [21]. The plant has been reported to possess bioactive compounds which inhibits bacterial growth [22].

Fruits of *C. limon* (L.) contain natural chemical components such as phenolic compounds (mainly flavonoids) and other nutrients and non-nutrients (vitamins, minerals, dietary fiber, essential oils and carotenoids). The vitamin C and flavonoids are deemed to be responsible for the health promoting effects of *C. limon* due to their antioxidant properties [23-25]. Among the health benefits of *C. limon* are its value as anti-catarrh, promotes blood circulation, and a capillary protector. It is antihypertensive, antispasmodic, diuretic, applied to the skin and mucous membranes as antibacterial and antifungal.

Concoctions of roots of *C. ferruginea* and *C. limon* are used locally in the Ghanaian community for the treatment of breast cancer, wounds, dysentery, toothache, mouth and skin infections [16].

It is expected that phytochemicals with sufficient antimicrobial efficacy could be utilized for the treatment of bacterial infection. Due to the importance of medicinal value of these plants, the current study aims at determining the in vitro antioxidant and antimicrobial properties of the methanol root extract of *C ferruginea* and ethanol root extract of *C limon.* Most works on *C. limon* has concentrated on its fruits and leaves and those of *C. ferruginea* has been on the aqueous root extract.

2. Materials and Methods

2.1 Sample collection and identification

Roots of *C. ferruginea* and *C. limon* were collected from around Ahensan and Gazza Hostels around Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. The plant materials were taxonomically identified and authenticated at the Department of Herbal Medicine, KNUST by Dr. Asare.

2.2 Chemicals, reagents

All chemicals were purchased from Sigma Aldrich Co. Ltd, Irvine, U.K., except the standard drugs. The organic solvents were of analytical grade and procured from BDH Laboratory Supplies (England).

2.3 Extraction of Plant material

The roots of *C. ferruginea and C. limon* were thoroughly washed with running water and then distilled water. They were air dried for two weeks and pulverized and stored in a desiccator until analysis.

2.3.1 Methanol Extract

Cold maceration was used for the extraction. 100g of the roots of *C. ferruginea* was dissolved in 500mL of methanol. The samples were soaked in the solvents for 48hrs at room temperature. The ethanol extracts were then strained, the marc (the damp solid material) was pressed, and the combined liquids clarified by filtration. The solvent in the filtrate were evaporated using a rotary evaporator (BUCHI Rota vapor R - 114) to concentrate the extract at 60 °C. The extract was dried in an oven at 60 °C to solid residue.

2.3.2 Ethanol Extract

100g of the roots of *C. Limon* was dissolved in 500 mL of ethanol. It was then taken through the same procedure as in 2.3.1 to obtain the extract.

2.4 Preliminary Phytochemical Screening

The extracts obtained were phytochemically screened to assess the presence of phytoconstituents using a modification of the standard methods by Trease and Evans [26].

2.5 In vitro Antioxidant Assay

Total antioxidant and DPPH radical scavenging activities of the two extracts were assessed using the phosphomolybdenum and 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assays respectively.

Preparation of sample and reagent solution

0.2 g of each plant extract was weighed and distilled water was used to make 250 mL of 800 µg/mL stock solution. 500 µg/mL, 400 µg/mL, 300 µg/mL, 250 µg/mL, 200 µg/mL, 150 µg/mL, 100 µg/mL, 50µg/mL and 25 µg/mL were made through serial dilution. 800 µg/mL stock solution of ascorbic acid was prepared by weighing 0.2g of ascorbic acid and distilled water was used to top up to 250mL. 500µg/mL, 400µg/mL, 300µg/mL, 250 µg/mL, 200µg/mL, 150 µg/mL, 100 µg/mL, 50 µg/mL and 25 µg/mL were made through serial dilution**.**

Reagent solution was prepared with 0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate.

2.5.1 Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity of the methanol extract of *C. ferruginea* and ethanol extract *C. Limon* was evaluated by the method described by Prieto and colleagues[27]. Ascorbic acid was used as the standard antioxidant drug. The assay is based on the reduction of Mo^{+6} to Mo^{+5} by the extract and subsequent formation of green phosphate-molybdate complex at acidic ρH. A 3 mL extract of different concentration was mixed with 30mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes. The absorbance of the reaction mixture was measured at 695 nm in triplicate using a UV Visible spectrophotometer (LKB Biochrom, Cambridge, England, Model 4050) against a blank (a solution of every other solution excluding the extract and ascorbic acid) after cooling to room temperature. Water (3mL) in the place of extract was used as the blank. The antioxidant activity was expressed as ascorbic acid Equivalent (mg/g of extract). A plot of measured absorbance of ascorbic acids against the concentration of ascorbic acid was made to obtain a calibration curve. From the various absorbance of the extract solution, the ascorbic acid equivalent determined.

2.5.2 DPPH Free Radical Scavenging Assay

The radical scavenging activity of the extracts were determined using the 1, 1- diphenyl-2-picryl hydrazyl (DPPH) assay. 1 mL of 0.3mM methanol solution of DPPH was added to 3 mL of different extracts in methanol at different concentrations (5, 10, 15, 20, 25 and 30 µg/mL). 3mL of ascorbic acid solution of 200 µg/mL and 100 µg/mL were prepared by serial dilution. Here, various concentrations of extracts soluble in methanol were prepared by dilution method. The mixture was shaken and allow to stand in the dark for 30 minutes after which the absorbance was measured

at 517 nm using methanol as a blank. Each absorbance was read in triplicate and the mean value taken.
The antioxidant activity was computed using the formula;

$$\% \, inhibition = \frac{Ao - A}{Ao} \, x \, 100$$

Where, Ao = Absorbance of control
A = Absorbance of test solution

The negative control was a mixture of 3mL of methanol and 1 mL of methanol solution of DPPH. IC_{50} of the extract was determined from a graph of percentage inhibition against concentration.

2.6 Antimicrobial Activity determination

Agar well diffusion and minimum inhibitory concentration assays were employed to assess the antimicrobial activities of the extracts.

2.6.1 Microorganisms used

Five micro-organisms were used to evaluate the antimicrobial activities of the extracts. Two Gram-negative bacteria (*Escherichia coli and Salmonella typhi*), two Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) and one fungus (*Candida albicans*). All microbial strains were obtained from the Department of Pharmaceutical Microbiology, College of Health Science, Kwame Nkrumah University of Science and Technology, Ghana.

2.6.2 Agar well diffusion assay

The potency of the extracts to inhibit microbial growth was assessed using the agar well diffusion method described by Agyare and colleagues[28] with slight modification. The microbial isolates used were grown in Mueller Hinton agar. Media containing 9 mL of sterile molten nutrient agar and 0.1 mL of standardized inoculum of test organism was poured into petri dishes and allowed to solidify. Wells in the agar were made using a sterile 7-mm cork-borer. Five wells were bored on each plate 20 mm apart and labelled appropriately. One of the holes was created in the center of the plate where 10 µL, 0.1M ciprofloxacin was added as positive control. The remaining wells were then filled with 50µL of the serial dilutions (20, 10, 5, 2.5% w/v) of the extract. The overall setup was allowed to stand for 30 minutes and incubated at 37ºC for 24 hours. The experiments were performed in triplicate. The zones of growth inhibition were measured and the mean calculated. The results were recorded as mean ± standard deviation.

2.6.3 Minimum inhibitory concentration (MIC)

The broth dilution method described elsewhere [29, 30] with slight modification, was employed to assess the MIC of the crude methanol and ethanol extracts of *C. ferruginea* and *C. limon* respectively. Different concentrations of extracts and standard drug were prepared using serial dilutions in test tubes. 5 mL of nutrient broth was placed in the test tubes at the test concentrations for all test organisms. 4.8 mL of extract at the prepared concentrations was added followed by 0.2 mL of a suspension of test micro-organism. Tubes were incubated at 37°C for 24 h after which a 0.2 mL solution of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) was added to each well and well shaken. Microbial growth was indicated by wells that changed colour to purple. Wells that were unchanged (in colour) indicated inhibition of microbial growth by the extracts. The minimum concentration showing no growth of test organism were recorded for each test organism as MIC of each test organism. The experiment was done in triplicate and the mean MIC calculated. The results were recorded as MIC ± standard deviation.

2.7 Thin Layer Chromatography (TLC)

The number of components present in the extracts were determined by the analytical and preparative TLC method. Normal phase pre-coated TLC sheets (20cm × 20cm with 0.2mm thickness, silica gel) were obtained commercially. A 100 mL mixture chloroform, ethyl acetate and methanol at different ratios (3:2:1 v/v/v; 3:1:1 v/v/v; 2:1:1 v/v/v) was tried as the mobile phase. The chromatogram was ran and the developed plates were dried in an oven at 50 ºC. They were then removed and sprayed with para-anisaldehyde solution and then heated at a relatively high temperature. The retardation factor (R_f) of the eluted spots was calculated using equation (1).

$$Rf = \frac{distance \; travelled \; by \; sample}{distance \; travelled \; by \; solvent \; front} \qquad (1)$$

From the results a ratio of chloroform: ethyl acetate: methanol (3:1:1 v/v/v) solution was found to give a good separation. This solvent system was then adapted and used in preparative TLC. TLC plates were dried and activated in an oven for 30 minutes at 105 ºC. After running the chromatogram, the plates were sprayed with para-anisaldehyde solution and dried in an oven at relatively high temperature for the pink to yellow colour of the background to develop. Each spot with slightly varying colour was marked with a pencil and the retention factor determined.

2.8 FTIR Analysis

The scrapped samples from the preparative thin layer chromatogram were dissolved in methanol. It was then centrifuged using Eppendorf Centrifuge 5415 C at a speed of 3000 rate per minute (rpm) for 10 minutes and the supernatant collected and filtered to remove the silica. The supernatant was subjected to FTIR analysis using Perkin Elmer Spectrum Version 10.03.09, Spectrum 2.

3. Results and Discussions

3.1 Phytochemical Screening

Phytochemicals are plant derived compounds that are responsible for most of the disease protection from consumption of plant based diets and concoctions [31]. The phytochemical screening showed the presence of various phytochemicals in the extracts of both *C. ferruginea* and *C. Limon*. The results are given in Table 1.

Table 1: Phytochemical constituents of methanolic extract of *C. ferruginea* and ethanolic extract of *C. limon*.

Phytochemical	*C. ferruginea*	*C. Limon*
Saponins	+	+
anthraquinones	+	-
glycosides	+	+
flavonoids	+	+
alkaloids	+	+
tannins	+	+
Coumarins	-	-

+ means present, - means absent

The extracts of both plants had five phytochemicals in common, that is saponins, tannins, glycosides, flavonoids and

alkanoids. The presence of flavonoids in C. *limon* is confirmed from studies that indicate that more than sixty individual flavonoids have been identified in Citrus sp., and most of them can be classified into flavanones, flavones and flavonols [32]. Ishola and Ashorobi [18] identified alkaloids, flavonoids, saponins and glycosides as the major constituents in the root extract of *C. ferruginea*, corroborating the results obtained of this work. However, *C. ferruginea* contained anthraquinones which were absent in *C. limon*. Coumarins were found to be absent in both extracts. Phenolic compounds such as flavonoids and tannins are known to have high antioxidant properties [33]. Free radical scavenging activity is likely carried out by flavonoids and tannins [34]. The presence of these phytochemicals in the extracts of the two plants indicate that they will play a key role in the prevention of various degenerative diseases as a result of their possession of antioxidant properties [35].

3.2 In vitro antioxidant capacity

Antioxidant capacity assays may be comprehensively classified as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. SET assays measure the capacity of an antioxidant in the reduction of an oxidant which changes colour when reduced. Other SET assays like Cupric ion reducing capacity assay (CUPRAC), Ferric ion reducing antioxidant power assay (FRAP) are also used[36]. The *in vitro* antioxidant capacity was accessed by using Total antioxidant capacity assay and the DPPH radical scavenging assay.

3.2.1 Total antioxidant capacity (TAC) assay

The phosphomolybdenum assay was used to evaluate the total antioxidant capacity of the methanol extract of *C. ferruginea* and ethanol extract of *C. limon*. Total antioxidant capacity is the ability of an extract or herbal matrix to delay oxidative stress in a controlled system. The TAC results are reported in Table 2.

Table 2: Total Antioxidants Capacity Test Results

Concentration (mg/mL)	TAC (mgAAE/g) ± S.D.	
	C. ferruginea	*C. limon*
0.025	0.711 ± 0.01	0.453 ± 0.02
0.050	0.871 ± 0.05	0.516 ± 0.04
0.100	0.951 ± 0.10	0.535 ± 0.02
0.150	1.132 ± 0.08	0.596 ± 0.01
0.200	1.332 ± 0.11	0.646 ± 0.09
0.250	1.515 ± 0.06	0.695 ± 0.07
0.300	1.767 ± 0.01	0.693 ± 0.12

TAC-Total Antioxidant Capacity AAE – ascorbic acid equivalent

The phosphomolybdenum (PM) assay is a type of SET based assay. PM assay, a quantitative method, is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of a bluish green colored phosphate/Mo (V) complex at acid pH [27]. Unlike CUPRAC and FRAP, PM occurs without induction of free metal ions solution so it shows uniqueness among *in vitro* antioxidant assays. The results were recorded as mgAAE/g ± standard deviation.

Generally, the TAC increased with increasing concentration of the extracts. At all levels of concentration of the extracts, the TAC were higher for *C. ferruginea* than for C. limon, indicating that *C. ferruginea* extract possesses a higher antioxidant capacity than the *C. limon* extract. However, both extracts exhibited appreciable antioxidant capacities.

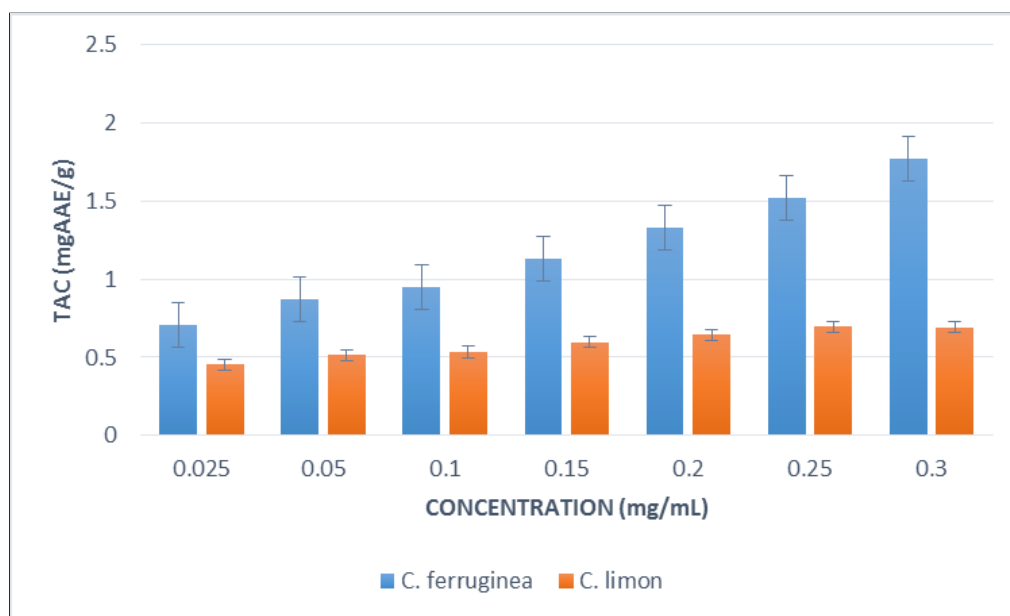

Fig 1: Total Antioxidant Capacity of the methanol extract of *C. ferruginea* and methanol extract of *C. limon*.

3.2.2 DPPH Radical Scavenging Assay

Ascorbic acid was utilized as the standard for the DPPH scavenging assay. Figure 2 depicts the DPPH scavenging capacities of the extracts of *C. ferruginea* and *C. limon*, and the standard drug.

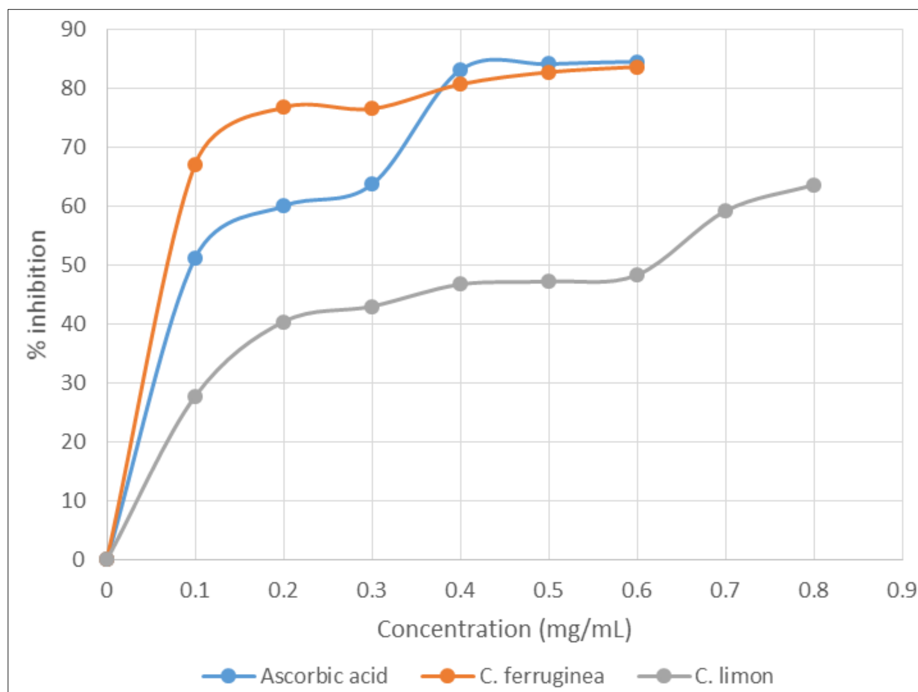

Fig 2: DPPH radical scavenging activity of methanol extracts of *C. ferruginea,* ethanol extracts of *C. limon* and ascorbic acid.

DPPH scavenging activity gives an indication that an extract has a potential antioxidant. The potential antioxidants in the extract react with the DPPH by donating hydrogen to the DPPH free radical in order to remove odd electron, hence the capacity to scavenge free radicals. DPPH is a stable nitrogen centered free radical which is violet in colour but which changes to yellow colour upon reduction by either hydrogen or electron reception. Generally, the percent inhibition of the extracts and standard drug increased with increasing concentration as depicted in figure 2. The methanol extract of *C. ferruginea* exhibited the highest percent inhibition between the concentration range of 0.00 to 0.3 mg/mL, whereas the standard showed the highest inhibition beyond this concentration range. *C. limon* exhibited the lowest inhibition at all concentrations.

The IC_{50} is the half maximal inhibitory concentration of the extract or standard drug that is able to inhibit or scavenge 50% of the oxidants. The IC_{50} values were obtained from the graph of percent inhibition against concentration. The IC_{50} values for ascorbic acid, *C. ferruginea* and *C. limon* were respectively 0.094 mg/mL, 0.069 mg/mL and 0.618 mg/mL. The lower the IC_{50}, the better the free radical scavenging activity[37]. The IC_{50} values obtained from the antioxidant assays showed that the extracts had potent scavenging activities on DPPH radical. Methanol extract of *C. ferruginea* showed better scavenging capacity than that of *C. limon*. The results revealed that C. *ferruginea* extract had higher antioxidant activity than the ascorbic acid reference solution used.

Both extracts showed varying antioxidant activity with methanol roots extract of *C. ferruginea* having high antioxidant activity in the Total Antioxidant Capacity (TAC) assay as well as DPPH free radical scavenging assay.

3.3 Antimicrobial capacity

3.3.1 Agar well diffusion test

All two plant extracts exhibited antimicrobial activity against test organism to various extents. *C. ferruginea* showed moderately high antimicrobial activity than *C. limon* ethanol extract. Clear zones of inhibition around wells indicated microbial inhibition and were thus measured as zones of inhibition reported in millimeters.

Table 3: Mean zones of inhibition (ZI) for extracts of *C. ferruginea, C. limon* and standard drug ciprofloxacin in agar well diffusion assay

Sample	Concentration (% w/v)	Mean zone of inhibition (mm)				
		S. aureus	*S. typhi*	*B. subtilis*	*E. coli*	*C. albicans*
Ciprofloxacin	0.01	25.5 ± 0.2	25.5 ± 0.1	21.5 ± 0.3	33.0 ± 0.2	32.5 ± 0.2
C. ferruginea	20.00	18.50 ± 0.1	18.50 ± 0.3	20.00 ± 0.2	20.00 ± 0.2	17.00 ± 0.1
	10.00	15.00 ± 0.1	13.00 ± 0.2	16.00 ± 0.1	15.00 ± 0.1	14.50 ± 0.1
	5.00	13.00 ± 0.3	NA	14.50 ± 0.2	13.00 ± 0.3	11.00 ± 0.3
	2.50	NA	NA	12.00 ± 0.1	11.00 ± 0.1	10.00 ± 0.1
C. limon	20.00	NA	14.00 ± 0.4	12.00 ± 0.2	14.00 ± 0.3	15.00 ± 0.2
	10.00	NA	12.00 ± 0.2	NA	NA	12.00 ± 0.2
	5.00	NA	NA	NA	NA	NA
	2.50	NA	NA	NA	NA	NA

NA – not active

The antimicrobial activity of methanol extract of *C. ferruginea* and ethanol extracts of *C. limon* was evaluated against four strains of bacteria and one fungus. The results are given in Table 3. *C. ferruginea* extract recorded its highest ZI of 20.00 mm against the Gram-negative bacteria *E. coli* and Gram-positive bacteria *B. subtilis. The* extract *of C. ferruginea* was active against all test organisms with ZI ranging from 10 mm – 20 mm. The mean ZI increased with

increasing concentration of extract. *C. ferruginea* showed activity at all tested concentrations for three of the test organisms, *E. coli*, *C. albicans* and *B. subtilis.* The extract of *C. limon* recorded zones of inhibition ranging from 12 –15 mm. It recorded its highest ZI of 15 mm against the fungus *C. albicans*. Remarkably, *C. limon* was inactive towards the *S. aureus* at all the tested concentrations. *C. limon* showed no activity at concentrations of 10 and 5 % w/v for all tested organisms. *B. subtilis* was found to be the most susceptible to the extract from *C. ferruginea* whilst *C. albicans* was the most susceptible to the *C. limon* extract. The standard drug ciprofloxacin also recorded a ZI ranging between 25.50 – 33.00 mm at concentration of 0.01 % w/v. All tested organisms were susceptible to the standard drug with the gram-negative bacteria *E. coli* showing the highest susceptibility. Both extracts showed activity against the fungus *C. albicans*.

3.3.2 Minimum Inhibitory concentration (MIC)

Table 4: Results of MIC for the extracts and standard drug (Amoxicillin)

Test Organisms	Minimum Inhibitory Concentration (mg/mL)		
	C. ferruginea	*C. limon*	Amoxicillin
S. aureus	5.0 ± 0.01	5.0 ± 0.02	-
S. typhi	10.0 ± 0.03	10.0 ± 0.17	5.0 ± 0.13
C. albicans	5.0 ± 0.15	10.0 ± 0.16	5.0 ± 0.08
B. subtillis	5.0 ± 0.02	10.0 ± 0.04	5.0 ± 0.14
E. coli	5.0 ± 0.21	5.0 ± 0.05	2.5 ± 0.01

The extracts were found to be appreciably potent as they had low MICs, as depicted in Table 4. The MICs of the extracts ranged between 5.0 and 10.0 mg/mL, whereas those of the standard drug ranged from 2.5 to 5.0 mg/mL. At MIC of 5 mg/mL *C. ferruginea* extract was lethal and could inhibit microbial growth in four of the test organisms including *S. typhi*. At the same MIC of 5.0 mg/mL *C. limon* could inhibit microbial growth of only *S aureus* and *E. coli*. However, at the MIC of 10 mg/mL *C. ferruginea* was able to inhibit growth of *S. typhi* and *C. limon* also inhibit growth for the remaining microbes being *B. subtilis*, *S. typhi* and the fungal strain *C. albicans*. The standard drug, amoxicillin recorded a lower MIC of 2.5 mg/mL against *E. coli* as compared to the extracts which gave 5 mg/mL for both *C. ferruginea and C. limon*. At MIC of 5 mg/mL the amoxicillin inhibited growth of all the microbes except *S. aureus*.

A considerable measure of information exists on the antimicrobial activities of an extensive variety of extracts of plant origin. This is particularly helpful for occupants in rustic areas in developing countries who have restricted access to synthetic antimicrobial drugs. This study has therefore provided additional information on the health benefits of plant natural products. The results of this study have revealed that methanol and ethanol extracts of *C. ferruginea* and *C. limon* respectively contain promising antimicrobial leads. *C. ferruginea* extracts was active against all test organisms, thus the extract showed varying degree of inhibition against test organisms. The methanol extracts of *C. ferruginea* and ethanol extracts of *C. limon* displayed higher antimicrobial activities as evidenced by the relatively lower minimum inhibitory concentrations (MICs) (Table 4).

C. limon (lemon) is an important medicinal plant of the family Rutaceae. It is cultivated chiefly for its alkaloids, which are known to possess anticancer and antibacterial activities in crude extracts of different parts namely leaves, stem, root and flower of lemon against clinically important bacterial strains[38]. Also, citrus flavonoids have a broad scope of biological activity including anticancer, antibacterial, antifungal, antidiabetic and antiviral activities [39-40].

Food borne pathogens impose a significant burden of infections in the developing countries. *S.typhi*, *S. aureus* and *E. coli* are amongst the most common pathogens responsible for food poisoning and food related infections in many parts of the globe [41, 42]. *E. coli* is a naturally occurring bacteria in the intestinal tract of man. The securing of invasion components expands their capacity to adapt to new niches and their disease-causing capacities[43]. The extracts' MIC values against *E. coli* as compared to the standard drug amoxicillin, reveal that they possess good leads against the growth of *E. coli*. *C. limon* extracts inhibited the growth of test organisms at concentration of only 20% (%w/v) except for *S. aureus* in which the extract had no ZI. The result in this report proved that extract of *C. ferruginea* has a higher antimicrobial activity than *C. limon.* Hence, data gleaned from the study reveals the inhibitory growth potential of *C. ferruginea* and *C. limon* extracts and gives credence to the fact that medicinal plants serve useful and therapeutic purposes. Similarly, studies carried out by Akharaiyi and others indicated that the root of *C. ferruginea* have antimicrobial activity against some species of microorganisms [22].

3.4 Thin layer chromatography (TLC)

Separation of components of the root extracts were achieved using TLC. Table 5 gives the results of the TLC analysis.

Table 5: TLC results of extract showing various components and their retardation factor using chloroform, ethyl acetate, and methanol as mobile phase (3:1:1).

Components	*Mean Retardation factor*	
	C. ferruginea	*C. limon*
A	0.28 ± 0.12	0.22 ± 0.15
B	0.44 ± 0.14	0.26 ± 0.07
C	0.54 ± 0.08	0.48 ± 0.11
D	0.76 ± 0.10	0.83 ± 0.06

Four components were identified for the root extracts of both *C. ferruginea* and *C. limon*

The Rf of the components of *C. ferruginea* ranged between 0.28±0.12 and 0.76±0.10 whereas those of the components of *C. limon* ranged between 0.22±0.15 and 0.83±0.06. The number of spots indicating the separated components are fewer than the identified phytoconstituents identified to be present in each root extract. This means that some of the components co-eluted and it may be necessary to employ two dimensional TLC to achieve complete separation of the components. Six and five phytochemicals were respectively identified for *C. ferruginea* and *C. limon* whereas they both produced four spots in the TLC analysis. Ongoing research is on the separation of all the components present in the extract

that will lead to isolation, purification, identification and bioactivity determination of each component.

3.5 FTIR Analysis.

The FTIR analysis was performed on components of each root extract that was the most concentrated. Component A from each of *C. ferruginea* and *C. limon* were analysed and the results have been presented in figures 3 and 4 respectively.

FTIR

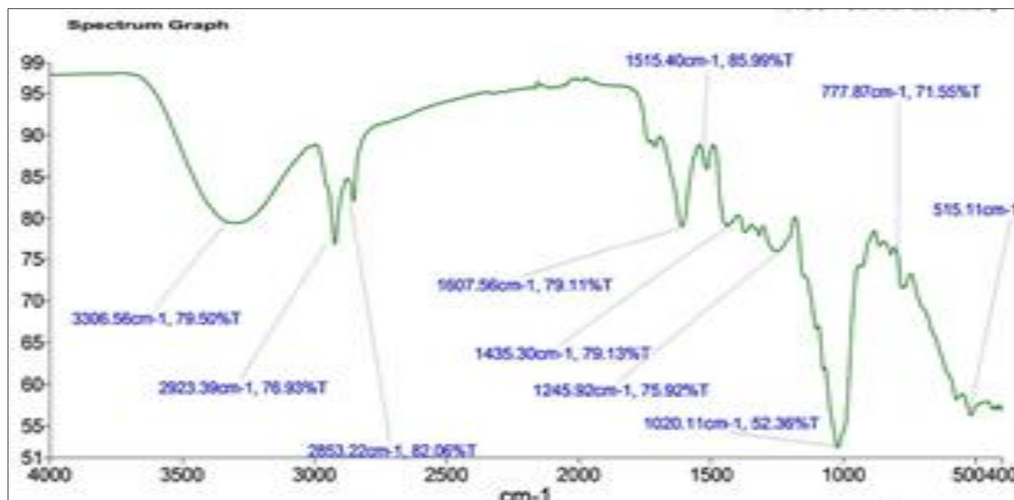

Fig 3: FTIR spectrum of Component … of *C. ferruginea* root extract

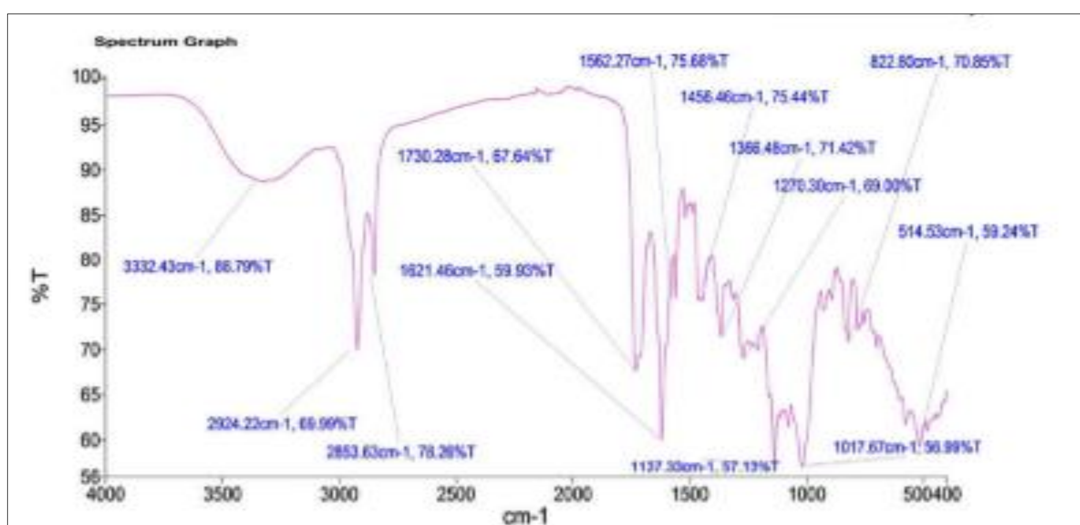

Fig 4: FTIR spectrum of Component ….. of *C. limon* root extract

Table 6: Crude extracts from *C. ferruginea* and *C. limon* samples in FT-IR analysis

***C. ferruginea* extract**		***C. limon* extract**	
Band position (cm^{-1})	**Functional Group**	**Band position (cm^{-1})**	**Functional Group**
3332.43	OH alcohol/phenol	3332.43	OH alcohol/phenol
2924.39	C-H stretch	2924.22	C-H stretch
2853.22	C-H stretch	1621.46	C=C aromatic/alkene
1607.56	C=C aromatic/alkene	1730.28	C=O stretch/carbonyl
1435.30	C-H bend	1137.33	C-O stretch
1020.11	C-C, C-N stretch	1017.67	C-N Aliphatic stretch

Similar wave numbers at 3332cm^{-1} were found in both extracts showing characteristic broad peaks representing alcohol/phenol (OH) functional group. Sharp peaks (Fig. 3 and Fig. 4) indicating asymmetric stretching of C-H showed around 2924 cm^{-1} and 2853 cm^{-1}. Aromatic/alkene carbon double bonds with wave numbers around 1607 and 1621 cm^{-1} showed in the spectrum of *C. ferruginea* and *C. limon* extracts respectively. The wave numbers 1730, 1435, 1137, 1020 and 1017 positions of the spectrums are characteristic C=O stretching, C-H bending, C-O stretch, C-C stretch and C-N aliphatic stretches respectively [44].

4. Conclusion

Concentrates from the methanol root extract and ethanol root extract of *C. ferruginea* and *C. limon* respectively demonstrated remarkable phytochemical diversity with the striking nearness of similar type of phytochemicals that incorporates tannins and flavonoids that accounts greatly for their observed antioxidant activities and alkaloids for their

antibacterial and antifungal activities. FTIR studies revealed the presence of alcohol or phenol carbonyl, ether, aromatic double bonds and aliphatic amine functional groups which may be therapeutically responsible for the medicinal use of these plants. The study demonstrated that *C. ferruginea* and *C. limon* extracts showed a variety of antibacterial, antifungal and antioxidant activities against human pathogens that would be of great importance in medicine. Both *C. ferruginea* and *C. limon* inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* at a minimum inhibitory concentration of 5 mg/mL whilst *Bacillus subtilis* and *Candida albicans* were also inhibited at an MIC value of 5 mg/mL by *C. ferruginea* extracts. *C. ferruginea* recorded a remarkable IC_{50} value of 0.063 mg/mL, greater than the standard ascorbic acid (0.094 mg/mL) used in the study. This study, gives *in vitro* data which may not be precisely reproduced *in vivo*. Additional studies directed at isolation, characterization and identification of novel antimicrobial and antioxidant compounds present in *C. ferruginea* and *C. limon* and *in vivo* studies that may validate the *in vitro* findings are underway.

5. References

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