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Research Article

Antibacterial Activities and Phytochemical Composition of Sunflower (*Tithonia diversifolia*) on Clinical Isolates of *Enterobacter cloacae*

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Abstract

This research work aimed at studying the antimicrobial activities of *Tithonia diversifolia* and its phytochemicals against eighteen (18) *Enterobacter cloacae*, a Gram-negative, facultative, anaerobic, rod-shaped, non-spore forming bacteria belonging to the family of *Enterobacteriaceae.* The increase of bacterial resistance specifically leading to treatment failures is directly responsible for the current increase in morbidity and mortality associated with bacterial infections. Resistance to β-lactams in *Enterobacteriaceae* is mainly (but not exclusively) caused by intrinsic and acquired β-lactamases. Two systems classify these enzymes, one based on the molecular structure and the other in their function. *Tithonia diversifolia* (Methanol and ethyl acetate extract) was then screened against 18 clinical samples from University College Hospital, Ibadan (UCH), Nigeria. The clinical isolates of *Enterobacter cloacae* were further reconfirmed and identified using Analytical Profile Index (API20E) before the determination of the antibacterial activity of the plant using the agar well diffusion. The study showed that ethyl acetate extracts *of Tithonia diversifolia leaf* showed little inhibitory effects against the tested organism compared to the methanol extract, which showed no observable inhibitory effect. This result further established the resistance of some of the species of *Enterobacteriaceae* (*E. aerogenes* and genus *klebsiella).*

However, due to the significant inactivity against the isolates by the methanol and ethyl acetate extract recorded even at higher concentrations, no further research was carried out on them. Therapeutic antibiotics (Rifampicin and Lincomycin) were used as control for the Minimum Inhibitory Concentration. Rifampicin against ten test isolates showed Minimum Inhibitory Concentration (MIC) values of 37.5 mg/mL (UCH 15, UCH 19), 75 mg/mL (UCH 1, UCH 4, UCH 5, UCH 12, UCH 17, UCH 18) and 150 mg/mL (UCH 10, UCH 16). The Phytochemical screening of extracts of the leaves of *Tithonia diversifolia* displayed showed the presence of saponin, glycoside, tannin, steroid, phenol, flavonoid and anthraquinone.

Keywords: Antimicrobial Activity; Minimum Inhibitory Concentration; Tithonia diversifolia; Phytochemicals

Introduction

The use of plants and its extract for medicinal purposes has been in use over the years and has also been the source of much useful therapy in both herbalism and folk medicine [1]. Medicinal plants contain a wide range of phytochemical compounds such as alkaloids, tannins, carotenoids, terpenoids, polyphenols, glycosides, vitamins, saponins [2]. Scientifically, it has been investigated that medicinal plants used in folklore remedies have attracted increased attention in the world of medicine in finding a lasting solutions to problems pertaining to health [3]. In an increasing search of new antimicrobial agent to cope with the microbial resistance to antibiotics, scientists are searching from different sources includ-

ing plants [4]. The genus *Tithonia,* belonging to the family Asteraceae, comprises eleven species, namely *Tithonia. exceisa, T. playlepsis, T. fruticose, T. tagitiflora, T. speciosa, T. scaberrima, T. glaberrima, T. ovata, T. tubiformis, T. diversifolia and T. rotundifolia* [4]. These species are of Mexican, Central American and Cuban origin [4]. *Tithonia diversifolia* popularly known as Mexican sunflower belongs to the member of the family Asteracease. It is an annual weed which grows aggressively in abandoned lands, roadsides, river banks and farmland [5]. *Tithonia diversifolia* a 2-5m tall plant that grows majorly in Mexico, Africa and parts of the world. The seeds are achens, 4-angled and 5m long, and are spread by wind [6]. *Enterobacter cloacae* (a gram negative, rod shaped bacteria) has been reported as an important opportunistic and multi resistant bacterial pathogen for humans during the last three decades in hospital wards, emerging as nosocomial infection [7]. *Enterobacter cloacea* have taken on clinical significance as opportunistic bacteria and have emerged as nosocomial pathogens from intensive care patients pathogenic, especially to those who are on mechanical ventilation [8]. Study shows that the pathogen is clinically important in contributing to bacteremia, endocarditis, septic arthritis, osteomyelitis, skin/soft tissue infections, lower respiratory tract infection and intra-abdominal infections [9]. *Enterobacter cloacae* is an important opportunistic pathogen known to cause nosocomial septicemia and urinary tract and respiratory tract infections. Reports of multidrug-resistant isolates have increased during the last decade, probably as a result of the extensive use of broad-spectrum antibiotics. In some patient populations, the derepressed production of the AmpC beta-lactamase is a mechanism of beta-lactam resistance in *E. cloacae* strains [10]. Nosocomial infections are most frequent from this organism, which means it may be contracted during hospitalization in hospital units such as the Intensive care unit (ICU).

Materials and Methods

Preparation of plant material

Fresh, mature leaves of the plant *(Tithonia diversifolia)* were collected within the premises of Male Halls of Residence of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria in October 2015. The leaves were rinsed with water to wash away sand particles before drying at room temperature for about 3 weeks after which it was blended into powder by a milling machine. The powdered leaf was later weighed to be 150 grams.

Specimen collection of *Enterobacter cloacae*

Clinical samples were obtained from patients using the medical laboratory service of University teaching hospital Ibadan, Nigeria. The clinical samples were collected from consented patients. The samples were then transported for identification to the laboratory unit of the department of Microbiology, Adekunle Ajasin University Akungba Akoko, Ondo State Nigeria. *Enterobacter cloacea* isolate were then reconfirmed using API20E.

Extraction process and preparation of *Tithonia diversifolia*

Extraction process was carried out with two solvents: methanol and ethyl acetate. The extraction was done by submerging 50 gram of the plant extracts into 1.5 liters of both methanol and ethyl acetate and allowed to soaked for about 2 weeks. The extracts were then evaporated using rotary evaporator and the solute underneath was scrapped and weighed. The extracts weighed 14.9 grams and 12 grams for methanol and ethyl acetate respectively after extraction. The extracts were then prepared for each solvent extract by dissolving 0.3 gram of extract and 0.5 volume of DMSO (dimethyl) and 5.2 mls sterile distilled water. Three concentration of extracts was prepared, which were 300 mg/ml, 150 mg/ml, 75 mg/ml.

Antimicrobial screening test using the agar well diffusion method

Methanol extract of *Tithonia diversifolia* leaf of Concentration 300 mg/ml, 150 mg/ml and 75 mg/ml ethyl acetate extract *of Tithonia diversifolia* leaf of the same concentration and antibiotics of concentration 300 mg/ml (rifampicin and lincomycin) used as the control was tested against 18 *Enterobacter cloacae* from different source of the body using agal well diffusion method and the zones of inhibition measured.

Mueller-Hinton agar was prepared by dissolving 36g of Mueller Hinton agar in 1000 ml of distilled water in a conical flask and dispensed into a sterile McCartney bottle before sterilizing in an autoclave at 121°C for 15 minutes. Then 0.2 ml of 24hrs culture of the isolate was dispensed into the cooled agar in the McCartney bottle before it was poured into sterile petri dishes and allowed to solidify. 0.8 mm sterile Cork borer was used to bore hole in the plate, then 1 ml of the therapeutic agents were seeded to fill the well and incubated at 37°C for 24 hours. After 24 hours of incubation, there

were significant clear zones of inhibition around the wells where the antibiotics used as control (rifampicin and lincomycin) were seeded than the wells where both methanol and ethyl acetate *Tithonia diversifolia* extract were seeded.

Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested microorganism in millimeter (mm). Each assay was carried out in duplicates and aseptically.

Minimum inhibitory concentration

Determination of minimum inhibitory concentration (MIC)

In determining the minimum inhibitory concentration (MIC) of the antibiotics that inhibited the growth of the organism, the agar-dilution method [11] was used. To 19 ml of molten Mueller-Hinton agar was added 1 ml of the therapeutic agent used (rifampicin and lincomycin).

The antibiotics (Rifampicin and Lincomycin) which were prepared to final concentrations of 150 mg/ml, 75 mg/ml, and 37.5 mg/ml for both antibiotics. The agar/antibiotics mixture was poured into sterile Petri dishes and allowed to set and were evenly streaked with an inoculating loopful of standardized overnight culture of the organism. After incubation for 24 hours at 37°C, the plates were examined for presence or absence of growth. The lowest concentration that prevented the growth of each organism was noted as the minimum inhibitory concentration.

Protocol of phytochemical analysis of *Tithonia diversifolia* lea: Qualitative method of analyses

Preliminary test/Preparation test

Plant filtrates were prepared by boiling 20g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrates were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyaonosides [12].

- **Test for alkaloids:** About 0.2gram was warmed with 2% of H2SO4 for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicates the presence of Alkaloids [12].
- **Test for tannins:** One milliliter of the filtrate was mixed with 2ml of FeCl. A dark green color indicated a positive test for the tannins [12].
- **Test for saponins:** One milliliter of the plant filtrate was diluted with 2 ml of distilled water; the mixture was vigorously shaken and left to stand for l0 minutes during which time, the development of foam on the surface of the mixture lasting for more than 10 mm, indicates the presence of saponins [12].
- **Test for anthraquinones:** One milliliter of the plant filtrate was shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10% (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test [12].
- **Test for anthocyanosides:** One milliliter of the plant filtrate was mixed with 5 ml of dilute HCI; a pale pink colour indicates the positive test [12].
- **Test for flavonoid:** One milliliter of plant filtrate was mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1ml of the plant filtrate were mixed with 2 ml of dilute NaOH; a golden yellow color indicated the presence of flavonoids [12].
- **Test for reducing sugars:** One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown color with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars [12].
- **Test for cyanogenic glucosides:** This was carried out subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil [12].
- **Test for cardiac glucosides:** Legal test and the killer-killiani was adopted, 0.5g of the extract were added to 2 ml of acetic anhydrate plus H2S04 [12].

Quantitative method analyses of *Tithonia diversifolia*

- **Saponins:** About 20 grams each of dried plant samples were ground and put into a conical flask after which 100 ml of 20% aqueous ethanol were added. The mixture was heated using a hot water bath. At about 55°C, for 4 hours with continuous stir ring, after which the mixture was filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer was recov-

ered while the ether layer was discarded. The purification process was repeated three times 60 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material [12].

- **Flavonoids:** About 10g of the plant sample were extracted repeatedly with 100 ml of 80 aqueous methanol, at room temperature. The whole solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weight [12].
- **Cardiac glucosides:** Legal test and the killer-Killani was adopted, 0.5g of the extract were added to 2 ml of acetic anhydrate plus H_2S0_4 [12].
- **Tannins:** About 500 ug of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric, flask and made up to the marked level [12].
- Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 MHcl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract [12].
- **Alkaloids:** Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then be added, the reaction mixture was covered and allowed to stand for 4 hours. These were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation is complete. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass [12].
- **Phlobatannins:** About 0.5 grams of each plant extracts were dissolved in distilled water and filtered. The filtrates were boiled in 2% HC1, red precipitate show the present of phlobatannins [12].

Determination of proximate analysis of *Tithonia diversifolia*

The proximate parameters (moisture, dry matter, ash, crude fats, proteins and fibers, nitrogen, carbohydrates and energy values) were determined using Association of Official Analytical Chemists Methods.

Determination of moisture

Content was done by drying samples in oven (Wise Ven, WON-50, Korea) at 110°C until constant weight was attained.

Determination of nitrogen content

Nitrogen estimation was carried out by the micro-Kjeldahl (BUCHI, Kjel Flex K-360, Switzerland) method with some modification.

Determination of protein content

The crude proteins were subsequently calculated by multiplying the nitrogen content by a factor of 6.25. The energy value estimation was done by summing the multiplied values for crude protein [12].

Determination of crude fat and carbohydrate content

Crude fat and carbohydrate respectively a Water Factors. Crude fats were determined by Soxhlet apparatus using n-hexane as a solvent [12].

Determination of ash value

The ash values were obtained by heating samples at 550 $^{\circ}C$ in a muffle furnace(Wise Therm, FHP-03, Korea) for 3 hours.

Determination of carbohydrate content

The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash content and crude fat from the total dry matter.

Determination of fibre content crude

Fiber was estimated by acid-base digestion with 1.25% H_2SO_4 (v/v) and 1.25%NaOH (w/v) solutions.

Determination of minerals by chemical analysis of phytochemicals

The ground samples were sieved with a 2mm rubber sieve and 2g of each of the plant samples were subjected to dry ash in a well cleaned porcelain crucible at 550°C in a muffle furnace. The resul-

tant ash was dissolved in 5 ml of HNO_3/H_2O_2 (1:1) and heated gently on hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100 ml volumetric flask by filtration through a what man filter paper and the volume was made to mark with de ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer (AAS). Concentration of each element was calculated on percentage of dry matter [12].

Results

Medicinal plants are gifts of nature used to cure diseases among human beings. The World Health Organization estimates that about 80% of the world population, settled in regions with low development and with low income makes use of Traditional Medicine as an alternative to attend to a variety of health problems. This is because pharmaceuticals currently available are very much expensive for most of the world's population [13]. The antimicrobial effect of *Tithonia diversifolia* was not really significant but the antibiotics used as control which are Rifampicin and Lincomycin both showed significant effect on the tested *Enterobacter* isolates.

Table 1 shows the phytochemicals present in *Tithonia diversifolia.* They include glycosides, steroids, phenol, tannins and saponins while alkaloids and anthraquinone tested negative. The phytochemical screening results of *Tithonia diversifolia* showed the presence of some antimicrobial constituents which includes phenols, tannins, saponins, glycosides and steroid as shown in table 1, these phytochemicals present in *Tithonia diversifolia* research has been previously investigated, having the aforementioned phytochemicals, having antibacterial activity [14]. Also, the mineral content analysis revealed the presence of essential minerals such as potassium, calcium, magnesium, iron, copper, zinc, manganese, sodium, this shows that *T. diversifolia has,* mineral constituents that has positive influence on human's health. The tested isolates *(Enterobacter cloacae)* were not susceptible to methanol *extract of Tithonia diversifolia* showing no significant antimicrobial activity even when the highest concentration (300 mg/mL) was used, (as shown in table 5). This similar result was previously stated as the outcome in the researched work by Dewole and Oni 2013 [15].

Sample	Alkaloid	Glycoside	Steroid	Anthraquinone	Phenol	Tannins	Saponin	Flavonoid
SUN	-ve	+ve	+ve	-ve	+ve	+ve	+ve	ND

Table 1: Qualitative analysis of the phytochemical screening of *Tithonia diversifolia.*

Keywords

SUN - Sunflower (*Tithonia diversifolia)* mg/l00g).

Ethyl acetate extract zones of inhibition ranges from 0 ± 0.0 mm to 12 ± 0.5 mm, of concentration 300 mg/mL, there were little antimicrobial effect against only isolates (UCH 6, UCH 10, UCH 11, UCH 12, UCH 14 and UCH 18), having 7 ± 0.5 mm, 12 ± 0.5 mm, l2 ± 0.5 mm,9 ± 0.5 mm, 11 ± 0.5 mm and 8 ± 0.5 mm zones of inhibition respectively, also 150 mg/mL concentration of ethyl acetate had zone of inhibition of 6 ± 0.0 mm, 10 ± 0.5 mm, 1 0 ± 0.5 mm, 6 ± 0.0 mm, 9 ± 0.5 mm and 6 ± 0.5 mm against isolates (UCH 6,UCH 10, UCH 11, UCH 12, UCH 14 and UCH 18) respectively while 75 mg/mL of *Tithonia diversifolia* ethyl acetate had zones of inhibition of 3 ± 0.5 mm, 9 ± 0.5 mm, 5 ± 0.5 mm, 4 ± 0.5 mm, 6 ± 0.5 mm and 4 ± 0,0 mm against isolates which 300 mg/ mL and 150 mg/mL extract showed inhibition.

Discussion and Conclusion

Plants produce a wide variety of phytochemical constituents, which are secondary metabolites and are used either directly or indirectly in the pharmaceutical industry. For centuries, man has effectively used various components of plants or their extracts for the treatment of many diseases, including bacterial infection [16]. Today the increase of resistance to antibiotics propels the search of new drugs to combat resistant microorganisms. Therefore, species commonly used as herbal medicine appear biologically active components isolated from plants secondary metabolites and their potential exert antimicrobial activities [17].

The phytochemical screening results of *Tithonia diversifolia* showed the presence of some antimicrobial constituents which in-

cludes phenols, tannins, saponins, glycosides and steroid (Table 1). Also, the mineral content analysis revealed the presence of essential minerals such as potassium, calcium, magnesium, iron, copper, zinc, manganese, sodium, as also stated by Omolola in her research [18]. As shown in table 2 (Quantitative analysis of minerals present in *Tithonia diversifolia)* the result reveals the presence of glycoside, steroid, phenol, tannins saponin and flavonoid except alkaloids and anthraquinone. *Enterobacter cloacae* showed resistant to ethyl acetate and methanol extract of *Tithonia diversifolia* even at high concentration.

Also, it has been reported that *Enterobacter cloacae* is naturally resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin and cefoxitin [7]. This study showed that clinical isolates of *Enterobacter cloacae* are highly resistant to both methanol and ethyl acetate extract of *Tithonia diversifolia, this* could be as a result of the of the extracts used. According to reports, the ethanolic extract of *Tithonia diversifolia* leaf extract showed the highest average zone of inhibition against *Staphylococcus epidermidis* (36.80 mm) followed by *Enterobacter aerogenes* (31.20 mm), *Streptococcus α-hemolytic* (29.60 mm), *Bacillus cereus* (26.2 mm), *Escherichia coli* (10.40 mm) [19].

Plant Sample	Na	K	Ca	Mg	Zn	Fe	Pb	Cu	Mn	P
SUN_1	20.34	24.34	20.72	23.18	19.82	5.95	ND	0.02	5.36	26.91
SUN_2	20.40	19.70	20.74	23.50	19.20	5.00	ND	0.02	5.31	25.85

Table 2: Quantitative analyses of minerals present *in Tithonia diversifolia.*

Keywords

SUN - Sunflower (*Tithonia diversifolia)* mg/l00g.

Comparing both the methanol and ethyl acetate antimicrobial results to the antibiotics used as the control which are rifampicin and lincomycin, from this study, it can be inferred that the purity characteristics of the antibiotics used as control made it to show greater zone of inhibition as against the impure plant extract. The antimicrobial activity was not significant compared to the control used, Lincomycin and Rifampicin. Only the chemotherapeutic agents Rifampicin (300 mg/mL) and Lincomycin (300 mg/mL) used as controls showed activity against the isolates with average diameter zones of inhibition as 19mm for Lincomycin and 15 mm for Rifampicin. As such only the chemotherapeutic agent lincomycin (zone of inhibition 19 mm) was used for further studies. The minimum inhibitory concentration (MIC) assay was done employing the agar dilution method and using the two control chemotherapeutic agent used (Rifampicin and Lincomycin). Ten *Enterobacter cloacae* that showed zone of inhibition was used for Rifampicin minimum inhibitory concentration assay (MIC) of isolates (UCH 1, UCH 4, UCH 5, UCH 9, UCH 10, UCH 12, UCH 15, UCH 16, UCH 17 and UCH 18). 37.5 mg/mL was recorded as the minimum inhibitory concentration value for isolate UCH 15 and UCH 9, 150 mg/mL concentration was recorded as the minimum inhibitory concentration value of isolate (UCH 10 and UCH 16) while 75 mg/mL was recorded as the minimum concentration value for the remaining tested isolates. The minimum inhibitory concentration (MIC) assay of Lincomycin was conducted for five susceptible *Enterobacter cloacae* isolates (UCH 9, UCH 13, UCH 14, UCH 17 and UCH 18) having a minimum inhibitory concentration value of 75 mg/L (As shown in table 6).

S/N	% Ash	% MC	% CP	Fat %	Fibre %	% CHO
SUN1	11.69	9.20	14.87	6.25	11.72	41.17
SUN 2	10.67	8.28	11.33	6.00	12.45	46.60

Table 3: Quantitative analysis of proximate nutrient composition of plant extract.

Extracts Results in Percentage (%).

Keywords

SUN= Sun flower (*Tithonia diversifolia*).

Keys: MC= Moisture contents; CP = Crude Protein; CHO= Carbohydrate.

Isolates	Methanol extract			Ethyl acetate extract			Control (Antibiotics)	
	Concentrations							
	300 mg/L	150 mg/ ML	75mg/mL	300 mg/ mL	150 mg/mL	75 mg/m L	Rifampicin 300mg/mL	Lincomycin 300mg/mL
UCH 1	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.5	0 ± 0.5	28 ± 0.5	0 ± 0.0
UCH 2	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.5	0 ± 0.5	0 ± 0.0	12 ± 0.5	0 ± 0.5
UCH3	0 ± 0.5	0 ± 0.5	0 ± 0.0	0 ± 0.5	0 ± 0.0	0 ± 0.5	0 ± 0.5	0 ± 0.5
UCH 4	3 ± 0.5	0 ± 0.5	0 ± 0.0	6 ± 0.5	3 ± 0.0	0 ± 0.5	26 ± 0.0	8 ± 0.5
UCH 5	6 ± 0.0	2 ± 0.0	0 ± 0.0	0 ± 0.0	7 ± 0.0	0 ± 0.0	24 ± 0.0	28 ± 0.0
UCH 6	7 ± 0.5	5 ± 0.5	0 ± 0.0	7 ± 0.5	6 ± 0.0	3 ± 0.5	0 ± 0.0	3 ± 0.0
UCH 7	0 ± 0.0	0 ± 0.0	0 ± 0.0	2 ± 0.5	0 ± 0.5	0 ± 0.0	2 ± 0.0	14 ± 0.5
UCH 8	0 ± 0.5	0 ± 0.0	0 ± 0.5	0 ± 0.5	0 ± 0.0	0 ± 0.5	0 ± 0.5	4 ± 0.0
UCH 9	0 ± 0.0	0 ± 0.5	0 ± 0.5	0 ± 0.5	0 ± 0.0	0 ± 0.5	25 ± 0.5	25 ± 0.5
UCH 10	0 ± 0.0	0 ± 0,5	0 ± 0.0	12 ± 0.5	10 ± 0.0	9 ± 0.5	25 ± 0.5	0 ± 0.5
UCH 11	0 ± 0.5	0 ± 0.0	0 ± 0.5	12 ± 05	10 ± 0.5	5 ± 0.5	0 ± 0.0	26 ± 0.5
UCH 12	0 ± 0.5	0 ± 0.5	0 ± 0.0	9 ± 0.5	6 ± 0.0	4 ± 0.5	30 ± 0.5	26 ± 0.5
UCH 13	0 ± 0.0	0 ± 0.5	0 ± 0.5	0 ± 0.0	0 ± 0.0	0 ± 0.0	23 ± 0.5	26 ± 0.5
UCH 14	0 ± 0.5	0 ± 0.5	0 ± 0.5	11 ± 0.5	9 ± 0.5	6 ± 0.5	0 ± 0.0	26 ± 0.5
UCH 15	0 ± 0.0	0 ± 0.0	0 ± 0.5	0 ± 0.0	0 ± 0.5	0 ± 0.0	26 ± 0.5	0 ± 0.0
UCH 16	0 ± 0.5	0 ± 0.5	0 ± 0.0	0 ± 0.0	0 ± 0.5	0 ± 0.5	25 ± 0.5	0 ± 0.0
UCH 17	0 ± 0.0	0 ± 0.5	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	25 ± 0.5	20 ± 0.5
UCH 18	0 ± 0.5	0 ± 0.5	0 ± 0.0	8 ± 0.5	6 ± 0.5	4 ± 0.0	23 ± 0.5	20 ± 0.5

Table 4: Antibiogram sensitivity pattern of 18 *Enterobacter cloacae* clinical isolates to *Tithonia diversifolia* leaf extract. Diameter of zone of inhibition (mm).

Keywords

UCH (University Teaching Hospital Ibadan).

Isolate	UCH 1	UCH4	UCH5	UCH 15	UCH 10	UCH12	UCH 9	UCH16	UCH 17	UCH 18
Mic value	75 mg/ mL	75 mg/ ml	75 mg/ mL	37.5 mg/ mL	150 mg / mL	75 mg/ mL	37.5 mg/ mL	150 mg/ mL	75 mg/ml	75 mg/ mL

Table 5: The minimum inhibitory concentration of rifampicin against ten isolates *of Enterobacter cloacae* (UCH 1, UCH 4, UCH 5, UCH 15, UCH 10, UCH 12, UCH 9, UCH 16, UCH 17 and UCH 18).

Isolates	UCH 9	UCH 17	UCH 13	UCH 14	UCH 18
Mic Value	75mg/mL	75mg/mL	75mg/mL	NIL	NIL

Table 6: The minimum inhibitory concentration of lincomycin against isolate *of Enterobacter cloacae* UCH 9 UCH 17 UCH 13 UCH 14 and UCH 18.

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