Original Research Article

Preliminary phytochemical screening and antimicrobial activity of methanol extracts of *Usteria guineensis* and *Sphaerocoryne gracilipes* on common pathogens and isolates of Extended Spectrum Beta-Lactamases (ESBLs) producing *Escherichia coli*

Abstract

Antibiotic resistance of microbial pathogens has become a threat to public health, with observed increase in outbreak of infections, therapeutic failure, morbidity and mortality. This has caused an increasing research on medicinal plants as alternative potential therapeutic agents. In this study, antimicrobial activity of *Usteria guineensis* (UG) and *Sphaerocoryne gracilipes* (SG) was investigated on standard organisms and clinical isolates of extended spectrum beta lactamase (ESBL) producing *Escherichia coli.*

The leaves of *Usteria guineensis* and *Sphaerocoryne gracilipes* were extracted using methanol. Phytochemical analysis was carried out on the medicinal plants according to standard procedure. The clinical isolates were screened for the production of ESBL using double disc synergy test. Agar well diffusion method was used to determine antibacterial activity of the crude extracts at 100 and 25 mg/ml while Minimum Inhibitory Concentration (MIC) was assessed using broth micro-dilution method.

The two plants were found to contain cardiac glycoside, alkaloids and saponin on phytochemical analysis. Out of the fifteen *Escherichia coli,* eleven (80%) show the production of the ESBL. The crude extract of the plant samples showed antibacterial activity against ESBL *E. coli* with zone of inhibition ranging between 11 to 18 mm for *Usteria guineensis* and 12 to 15 mm for *Sphaerocoryne gracilipes*. The MIC of the crude extracts was ranging between 0.78 mg/ml to 50 mg/ml and MBC ranging from 12.5 to above 50 mg/ml for the ESBL producers and the standard strains.

Therefore, the plant *Usteria guineensis* have potential activity to be used in the treatment of infections caused by ESBL producing *E. coli* while the two plants can be developed as alternative therapeutic agent for the treatment of infections caused by *Staphylococcus aureus*

1. Introduction

The increased usage of beta-lactam antibiotics like penicillins and cephalosporins has produced an increase in resistance of bacteria in the family Enterobacteriaceae to many antibiotics of therapeutic importance[14, 17]. The primary mechanism of resistance to beta-lactam antibiotics is the formation of extended-spectrum beta-lactamases (ESBLs), which inactivate beta-lactam antibiotics. This is the primary cause of β-lactam antibiotic resistance among Enterobacteriaceae worldwide. ESBL-producing enterobacteria are common among the antibiotic-resistant bacteria that cause nosocomial and community-acquired illnesses [20].

Bacteria produce an enzyme called ESBLs that break down β-lactam drugs (except for cephamycins and carbapenems), thus allowing them to become resistant to extended-spectrum penicillin, cephalosporins, and monobactams. Beta-lactamase inhibitors, such as clavulanic acid, also act to prevent enzymatic hydrolysis of β-lactam drugs. Report on the development of resistance to extended spectrum cephalosporin by ESBL-producing Enterobacteriaceae has been on the rise [18], which requires serious attention. ESBLs have been found in Enterobacteriaceae, especially*Klebsiella* species and *E. coli*[8], and also among the non-lactose fermenters like*Pseudomonasaeruginosa* and *Acinetobacterbaumannii*. The gene responsible for the expression of antibiotic resistance is usually mediated by the chromosome or plasmid. Though ESBL is plasmid-mediated, it is easily spread across Enterobacteriaceae members; not just to beta-lactams, but also to other regularly used antibiotics such fluoroquinones, aminoglycosides, and sulphonamides. As a result, many patients require antibiotics of last resort, such as carbapenems [7]. Hence, there is need for routine surveillance for ESBL-production and also the rational choice of antibiotics for the treatment of infections and reduction in the burden of antimicrobial resistance in clinical settings [10].

In Africa and Asia, a majority of the populace still depends on traditional medicine for their primary healthcare needs [26] due to the growth in antibiotic resistance and the scarcity of conventional antibiotics. Plants have been used over time as a source of natural products for human health. Therefore,many medicinal plants have been investigated for their antimicrobial properties and some have been utilized as therapeutic alternatives [27, 2]. Secondary metabolites in plantssuch as saponins, phenolic compounds, flavonoids and alkaloids have

been identified as responsible for this antimicrobial and therapeutic properties [12, 25].The use of traditional medicine,commonly referred to as complementary and alternative medicine is expanding quickly in developed nations including United States, UK, Germany whereplant portion's active ingredients are used to treat diseases, prevent illness and manage cancer [13]. Moreover, many local medicinal plants of Ethiopia e.g. *Centellaasiatica* and Silybum *marianum* have shown good antibacterial activity against multidrug resistant (MDR) pathogens [15]. The onus rests on natural product scientists to search and discover more medicinal plant products or phytochemicals that can be used as alternative medicines in the treatment of diseases. Therefore, two Nigerian medicinal plants, with little or no report on phytochemical and pharmacological activities are hereby investigated for antibacterial activity against ESBL producing microbes represented by Escherichia coli of the family Enterobacteriaceae. This will also provide useful scientific information on the 'new' plants, especially phytochemical and antimicrobial.

Usteria guineensis Willd. (Loganiaceae) is a native plant to the tropical region of Africa and the only *Usteria* species in West Africa. The plant is found in the Southern Nigeria where it identified under the names 'Ukala' (Edo), 'Oporo' and 'Esinsin-ile' (Yoruba), where they use it for medicine and as fibres [6].According to, [11] the roots and leaves of*U. guineensis*are used to treat cough, parasitic skin infections and bronchitis.The Mende people of Sierra Leone use the roots to treat malaria by rubbing them on their foreheads, necks, and joints; but in Togo, the root decoction is used to cure gonorrhoea [23].

Sphaerocoryne gracilipes (Benth.) X.Guo & R.M.K Saunders (Annonaceae). Former name is Oxymitra longipedicellata (Baker f.) Sprague & Hutch. (Annonaceae). The native range of this species is Nigeria to West Central Tropical Africa. It is a climbing shrub. Petals are yellowish on the outside and purple on the interior at the base andit has scarlet fruits. The genus *Sphaerocoryne* Scheff. ex Ridl. (Annonaceae) is native to: Nigeria to Kenya, South Tropical Africa, Indo-China to Malesia. Two **species** of Sphaerocoryne are known from Africa, *S. gracilipes* in Nigeria and Central Africa *S. gracilis* in Zimbabwe and East Africa. The plant, *S. gracilipes* is 'novel' with no much work done on it and no information is available on its ethno-medicinal use [9].

Figure 1: ***Sphaerocoryne gracilipes*** **(Annonaceae)**

Figure 2: ***Usteria guineensis*** **(Loganiaceae)**

2. MATERIALS AND METHODS

2.1 Plant materials

The leaves of *Sphaerocoryne gracilipes* and *Usteria guineensis* were obtained from Ikire in Irewole Local Government of Osun State and was authenticated at Forestry Research Institute of Nigeria (FRIN), with deposited voucher specimen number FHI-114074 and FHI-114073, respectively

2.2 Extraction

The powdered leaves of *Sphaerocoryne gracilipes* (150 g) and *Usteria guineensis* (100 g) were weighed into a clean apparatus and soaked with enough methanol (extracting solvent). The soaked plant samples were shaken vigorously at intervals of 4 hours for 72 hours. The extracted matter was filtered using fresh cotton plug and then using filter paper (Whatman No. 1). The filtrate was concentrated using a rotary evaporator. The resulting crude extract of each solvent was weighed and stored in the refrigerator below 4^{o}C [24], until used for microbial assay.

2.3 Phytochemical screening for secondary metabolites

The extracts were screened for secondary metabolites using the powdered samples of the different plant parts. The tests were carried out in accordance with standard procedures[25].

2.3.1 Alkaloids

About 1 g each of the powdered leaf sample of *U. guineensis and S. gracilipes* was extracted with 10 ml HCl on water bath. The extracts were filtered and the pH was adjusted to 6.5 using Sodium Hydroxide solution. Drangendorff′s reagent, Meyer′s reagent and Wagner′s reagent was added to 3 ml of the filterate drop by drops. The test tubes were shaken and colour change was observed as follows: Drangendorff′s reagent(reddish brown), Wagner′s reagent (reddish brown) and Mayer′s reagent (creamy precipitate).

2.3.2 Anthraquinone Glycosides

About 0.2 g each of the powdered leaf samples of the plant extracts was placed in a dry clean test tube and 5 mls diluted sulphuric acid was added. The test tube was heated for 5 minutes and cooled. The contents were partitioned against the same volume of chloroform and the layers were allowed to separate. The chloroform layer was then carefully transferred to a clean test tube and then shaken together with 5 ml of 10% ammonium solution.A pink color observed in the aqueous layer indicate presence of anthraquinones.

2.3.3 Saponin Glycosides

10 ml of distilled water was added to about 1g of each powdered samples of the plant extracts in test tubes and heated for approximately 10 minutes. This was filtered while hot and the aqueous extract was used to demonstrate frothing by diluting 2 ml of the filterate to 10 ml with water and it was shaken. Formation of a persistent froth indicated presence of saponins.

2.3.4 Cardiac Glycosides.

Keller-Killiani Test

Exactly 0.3 ml of 10% of ferric chloride in 50% glacial acetic acid was added to a portion of the dried extract residue of the plants in clean test tubes. Then 2ml of conc. sulphuric acid was added to the side of the test tube. This produces another layer below the acetic acid layer. A brown ring formation at the interphase indicates the presence of deoxy-sugar.

Kedde Test

The dried residue was also mixed with 1 ml of 2% 3,5 dinitrobenzoic acid in ethanol. Then 5% sodium hydroxide solution was added to the solution. It was mixed thoroughly and the formation of brown purple color shows the presence of an unsaturated lactone ring.

2.3.5 Tannin

1g each of powdered plant samples was boiled for 5 minutes. Filteration was done and each filterate was made up to 10 ml volume; 5% ferric chloride solution was then added. A blue-black precipitate shows presence of tannin.

2.5 Test organisms

Fifteen (15) *Escherichia coli* isolates from urine samples were collected from Molecular Lab, Faculty of Pharmacy, University of Ibadan. *Escherichia coli* ATCC11175, *Bacillus subtilis* ATCC6633, *Salmonella typhimium* ATCC 14028, *Staphylococcus aureus* ATCC 29813and *Pseudomonas aeruginosa* ATCC 27853 used as reference were collected from Department of Pharmaceutical Microbiology Laboratory, University of Ibadan. They were all maintained on agar slants at 4˚C prior to use.

2.6 Detection of ESBL-producing Bacteria Using Double Disc Synergy Test

The detection of the ESBL production among the clinical isolates was determined by double disc synergy test using two third generation cephalosporin discs; ceftazidime and cefotaxime[10]. A fresh pure culture of *E. coli* was prepared and used to prepare a suspension of the test organism at the standard of 0.5 McFarland standard (1.0 x 10^8 cfu/ml). The prepared suspension of the test organisms was applied on the Mueller Hinton agar surface using sterile cotton. Next, the discs were put on the plates that had been infected. To enable accurate assessment of the diameter of the zone of inhibition, an augmentin disc was positioned in the center, and ceftazidime and cefotaxime discs were positioned 20 mm away from the clavulanic acid disc. After that, it was incubated for 18 to 24 hours at 37˚C. The tests were carried out in duplicates.

2.13 Antimicrobial Activity of Extracts

The procedures of agar well diffusion method [29] were followed. The Mueller Hinton agar was seeded with a standardized bacteria suspension (based on a turbidity of 0.5 McFarland standard, or 1 ×108 cfu/ml) using the agar well diffusion method. On the agar surface, consistent wells were punched using a sterile cork borer with a 9mm diameter. Using a sterile pasteur pipette, the crude extracts (25 and 100 mg/ml) were then added to the wells and left to diffuse. The positive and negative controls were represented by control wells that contained 10% DMSO4 and 10µg/ml of gentamycin, respectively. The tests were carried out in duplicates. The zones of inhibition were measured 24 hours after the bacterium plates were incubated at 37˚C.

2.14 Determination of Minimum Inhibitory Concentration (MIC) of Plant Extracts

The MIC was determined using broth microdilution technique following CLSI guideline[4]using broth dilution in 96-well micro-titer plate. The bacteria suspension havingturbidity equivalent to that of 0.5 McFarland Standard (1.5 x 10^8 cfu/mL) were inoculated into broth microdilution plates already containing different dilutions of the extracts (50 to 0.097 mg/mL) and control antibiotics (gentamycin) into100 µL of Mueller Hinton broth.The plates were incubated at 37 oC for 24 hours. The plates were examined for growth by the addition of tetrazolium salt. A change in colour to red or pink is indicative of growth of test organism. The lowest concentration of the antibiotics showing absence of growth is taken as the MIC. Two columns of the titre plate containing broth and antibiotics and broth alone were used as positive and negative control, respectively.

2.15 Determination of Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration was determined by inoculating freshly prepared Mueller Hinton broth in test tubes with inoculum from the wells showing no visible growth as indicated by no change in colour (in the MIC determination above). The tubes were then incubated at 37^0C for 24 hours. The lowest concentration of the extract showing absence of the growth is recorded as the MBC[1].

3. RESULTS

3.1 Extraction yield

The percentage yield of the plants was calculated with the formula: dry weight of extract/ dry weight of plant samples × 100. The yields obtained were 4.3% for *Usteria guineensis* which was lowerthan 5.9% for*Sphaerocoryne gracilipes*(Table 1).

The results of phytochemical screening, as shown in Table 2 revealed that the leaves of the two plants, though belonging to different family, have similar secondary metabolites, viz: saponin, alkaloid and cardiac glycoside.However, while*Usteria guineensis*was found to possess tannins, *Sphaerocoryne gracilipes*showed absence of tannins.

The results of ESBL production of the clinical isolates using double disc synergy test showed that specifically 73.33% of the *Escherichia coli* isolates were ESBL producers. Three of the *E. coli* were resistant to cefotaxime and ceftazidime, while the remaining *E. coli* did not show a ≥5 mm increase in inhibition zone of cephalosporin with Clavulanic acid (according to [4])(Table 3).

Antibacterial screening of the methanol extracts demonstrated a poor antibacterial activity on the gastrointestinal pathogens and standard isolates with the exception of *Staphylococcus aureus*. The concentration of the plant extracts at 25mg/mL and 100mg/mL showed little or no zones of inhibition compared to the gentamycin used as positive control. The diameters of zone of inhibition were between 11-15mm and 8-18mm at 25mg/mL and 100mg/mL of *U. guineensis* while the diameters obtained from *Sphaerocoryne gracilipes* was between 11-18mm and 12-19mm at concentrations 25 and 100mg/mL, respectively(Table 4).

The MIC value of both the methanolic leaf extract of *U. guineensis* and *S.* gracilipes were found to range between 0.78-12.5 mg/mL and 1.56-50.0mg/Ml, respectively while the MBC

ranged from 6.25 - 50mg/mL and 12.5- >50 mg/mL for *U. guineensis* and *S. gracilipes* on the common pathogens and clinical isolates. This result is presented in Table 5.

Table 1: Yield of plants' samples on extraction with methanol

Plants	Part used	Wt of Sample (g)	Wt of Extract (g)	Yield (%)
U. guineensis	Leaf	100.0	4.3	4.3
S. gracilipes	Leaf	150.0	8.9	5.9

Table 2: Phytochemical results for *U. guineensis* and *S. gracilipes*

Phytochemicals	U. guineensis	S. gracilipes
Alkaloids	+	+
Cardiac glycoside	+	+
Anthraquinone	-	-
Tannins	+	-
Saponins	+	+

Keys: + = present, - = absent

Table 3: Detection of synergy between Amoxicillin/ Clavulanic acid disc (30/10µg) and two third generation cephalosporin disc

Isolate	**Ceftazidime(30µg)**			**Cefotaxime(30µg)**		
	M	Mc	≥5mm	M	Mc	≥5mm
E1	14	28	14	16	30	14
E2	14	30	16	20	31	11
E3	10	22	12	16	28	12
E4	20	30	10	24	38	14
E5	-	-	-	-	-	-
E6	12	30	18	20	29	9
E7	15	24	9	16	15	1
E8	5	9	4	5	20	15
E9	-	-	-	-	-	-

E10	12	39	27	16	31	15
E11	12	24	12	20	32	12
E12	12	32	20	24	32	8
E13	30	32	2	42	42	0
E14	-	-	-	-	-	-
E15	18	30	12	20	30	10

KEYS: M= Diameter of Zone of inhibtion without Clavulanic acid disc Mc= Diameter of Zone of inhibtion with Clavulanic acid disc, - = No inhibition

Table 4: Antimicrobial activity of extracts on gatro-intestinal clinical isolates and common pathogens

	ZONES OF INHIBITION OFEXTRACTS AND CONTROLS(mm)					
Extracts	*Usteriaguineensis*		*Sphaerocorynegracilipes*		Gentamycin	DMSO
Isolates	25mg/ml	100mg/ml	25mg/ml	100mg/ml	10µg/ml	10%
E1	-	-	-	-	18	-
E2	-	-	-	-	17	-
E3	14	12	-	-	-	-
E4	-	-	-	-	18	-
E5	12	-	-	-	-	-
E6	-	-	-	-	-	-
E7	-	-	-	-	16	-
E8	12	-	-	-	-	-
E9	-	-	-	-	14	-
E10	-	-	-	-	-	-
E11	14	14	12	15	15	-
E12	11	11	11	12	13	-
E13	11	11	-	12	11	-
E14	14	14	12	15	-	-
E15	-	-	-	13	20	-
Ec	-	-	-	-	18	-
Sa	15	14	18	19	13	-
Pa	11	11	-	11	14	-
Bs	-	-	-	-	10	-
Stm	12	-	-	14	16	-

Keys: - = Not active, Ec= *Escherichia coli* ATCC11175, Bs= *Bacillus subtilis* ATCC6633, Stm= *Salmonella typhimium* ATCC 14028, Sa= *Staphylococcus aureus* ATCC 29813and Pa= *Pseudomonas aeruginosa* ATCC 27853

Table 5: MIC and MBC of plants' extracts on selected isolates and standard strains

	MIC (mg/ml)		**MBC (mg/ml)**	
ISOLATES	UG	SG	UG	SG
E2	6.25	50	12.5	50
E3	6.25	25	25	50
E4	12.5	12.5	25	25
E5	6.25	50	12.5	50
E6	12.5	12.5	25	50
E8	6.25	12.5	25	25
E9	25	12.5	12.5	25
E14	12.5	ND	25	ND
Ec	6.25	50	50	>50
Sa	0.78	1.56	50	>50
Pa	3.125	12.5	12.5	100
Bs	12.5	12.5	25	>50
Stm	12.5	12.5	50	>50

Keys: E: *Escherichia coli* isolates**,** Ec: *Escherichia coli* ATCC11175, Bs: *Bacillus subtilis* ATCC6633, Stm: *Salmonella typhimium* ATCC 14028, Sa: *Staphylococcus aureus* ATCC 29813,and Pa: *Pseudomonas aeruginosa* ATCC 27853, UG: *Usteria guineensis and* SG: *Sphaerocoryne gracilipes,* ND: Not determined

4. DISCUSSION

Bacteria pathogens are responsible for a large number of gastrointestinal tract infections with young children, the elderly and immunocompromised individuals at greater risk. Infections of the gastrointestinal tract have a significant impact on morbidity and mortality with success of treatment being threatened by the production of Beta Lactamases which inactivates most antibiotics [5]. Enterotoxigenic *Escherichia coli*in its pathophysiology colonises the upper GIT wherethey produce an enterotoxin that stimulates the mucosa cells to secrete fluid via an increase in intracellular cAMP which leads to diarrhoea. Invasive bacteria such as *Salmonella* also colonises the lower ileum, which may lead to the production of painful stools with blood [21]. This infection is usually prominent in areas with poor sanitation practices. The gastrointestinal pathogens used in this study were found to be ESBL producers as presented by double disk synergy test. Among the 15 *E. coli*, eleven showed positive results as ESBL which may have posed a serious threat to the use of antibiotics, rendering them ineffective. The three *E. coli* strainsthat showed no sensitivity to cefotaxime and ceftazidime may be

Multidrug resistant (MDR) and most likely will not respond to conventional treatment with the antibiotics. The emergence of this antibiotic resistance has been attributed to misuse of antibiotics [3].

However, methanolic leaf extract of *U. guineensis* showed zones of inhibition as wide as 11-15mm for most of the organisms and 11-14mm on the organisms at 25mg/mL and 100mg/mL. The MIC obtained for *S. aureus* strain was found to be 0.78mg/mL which is lower to the MIC value obtained for the ESBL-producing*E. coli,Klebsiella, Pseudomonas, Salmonella andBacillus* species used in this study which range from 3.125- 25mg/mL. On the other hand, the methanolic leaf extract of *S. gracilipes* had diameters of zones of inhibition ranging from 11-18 and 11-19mm at concentrations 25mg/ml and 100mg/mL with MIC ranging from 12.5-50mg/mL on most strains except for *S. aureus* which showed sensitivity to the crude extract at 1.56mg/mL. The two plant extracts can thus be used as a therapeutic alternative in the treatment of infections caused by *S. aureus. U.guineensis* had higher antibacterial activity than*S. gracilipes,*therefore may be more effective in the treatment of ESBL *E. coli* infections. The bactericidal activity of the plant extracts was obtained by calculating the MIC index (MBC/MIC), which was found to be 2 for *U. guineensis*(greater and more consistent than for *S. gracilipes*)for most of the tested organisms. The implication of this is that *U. guineensis*is bactericidal in action, unlike *S. gracilipes* with lower MIC index which implies that it is more of bacteriostatic in action [15].

The two plant extracts were found to have alkaloid, saponins and cardiac glycosides at concentrations which may be responsible for their antibacterial activity on *S. aureus.* The absence of tannins in *S. gracilipes*might have contributed to less activity of the extracts on the ESBL *E. coli* as tannins have been reported to possess antimicrobial activity, accoding to[22].

5. CONCLUSION

The extracts used in this study had fair activity on ESBL *E. coli,* the activity recorded on *U. guineensis* showed it has a fair potential in the treatment of ESBL *E. coli* infections. The result of this study supports the traditional application of *U. guineensis* and *S. gracilipes* to treat infections caused by *Staphylococcus aureus.* This proves that the plant can be used as an alternative therapeutic agent in the presence of alarming antimicrobial resistance.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

REFERENCES

1. Adeniyi, B. O. and Ayepola, O. O.(2006). The phytochemical screening and antimicrobial activity of leaf extracts of Eucalyptus camaldulensis and Eucalyptus torelliana (Myrtaceae). Research Journal of Medicinal Plants, 2006. 2 (1): 34 – 38
2. Adriana, B., Almodovarl, A. N., Pereiral, C. T., and Mariangela, T. (2007). Antimicrobial efficacy of Curcuma zedoaria extract as assessed by linear regression compared with commercial mouthrinses. Braz. J. Microbiol. 38: 440-445
3. Arturo, G., Marco, V., Eric, O. (2018). Current challenges in antibiotic stewardship in low and middle-income countries. *Current Treatment Options in Infectious Diseases* 10(3): 421-429.
4. Clinical and Laboratory Standards Institute (2014). *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement* (M100-S24)
5. Davidson, H., Christopher, G., and Roma, C. (2016). Intestinal Infections: Overview. International Encyclopedia of Public Health (Second Edition). 322-335
6. Emmanuel I.E. (2014). Annotated Checklist of Vascular Plants of Southern Nigeria - a quick reference guide to the *Vascular Plants of Southern Nigeria: a systemic approach*. Uniben Press, Benin City. 346p. pg 191.
7. Georgios, M. (2016) Carbapenem resistance: Overview of the problem and future perspectives. *Therapeutic advances in Infectious Diseases*. 3(1): 15- 21
    a. Health Organization. https://iris.who.int/handle/10665/312342.
8. Husna, A.; Rahman, M.M.; Badruzzaman, A.T.M.; Sikder, M.H.; Islam, M.R.; Rahman, M.T.; Alam, J.; Ashour, H.M.(2023). Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. *Biomedicines***2023**, *11*, 2937. https://doi.org/10.3390/biomedicines11112937
9. Hutchinson and Dalziel. (1954). Annonaceae. Flora of West Tropical Africa, 1:1
10. Idowu, P., Oguntifa, P., and Olaniran, O. (2020). Plasmid profile of Extended Spectrum beta lactamase (ESBL) producing multidrug resistant *Klebsiella* species from different clinical sources, *Afr. J. Med. Sci* 49
11. Iwu M.M (1993). Handbook of African Medicinal Plants. CRC Press. pg 43.

12. Iwu, M.W., Duncan, A. R. and Okunji, C. O. 1999. New antimicrobials of plant origin, In: *Perspectives on new crops and new uses,* J. Janick, (Ed.): 457-462
13. Jamsidi-kia, F., Zahra, L., and Hossein, A. (2018). Medicinal plants: Past History and Future Perspective. *Journal of Herbmed Pharmacology,* 7(1): 1-7
14. Jett B.D. and Ritchie D.J. (1995) In vitro activities of various ß-lactam antimicrobial agents against clinical isolates of Escherichia coli and Klebsiella spp resistant to oxyimino cephalosporins. Antimicrob. Agents Chemother. 39 (5) 1187-1190.
15. Manilal A, Sabu KR, Tsefaye A, Teshome T, Aklilu A, Seid M, Kayta G, Ayele AA, Idhayadhulla A. {2023) Antibacterial Activity Against Multidrug-Resistant Clinical Isolates of Nine Plants from Chencha, Southern Ethiopia. Infect Drug Resist. 2023 Apr 27;16:2519-2536. doi: 10.2147/IDR.S402244. PMID: 37138837; PMCID: PMC10150743.
16. Newman, D.J., Cragg, G.M. and Snader, K.M. (2003). Natural products as sources of new drugs over the period 1981–2002. Journal of Natural Products 66(7): 1022–1037.
17. Okeke, I.N., Aboderin, O.A., Byarugaba, D.K., Ojo, K.K. and Opintan, J.A. (2007). Growing problem of multidrug resistant enteric pathogens in Africa. Emerg. Infec. Dis. 13(11): 1640-1646.
18. Paterson, D. (2006) Resistance in gram-negative Bacteria: Enterobacteriaceae. *Am J Med.*, 119(6): 20–28.
19. Perez, C., Pauli, M. and Bazerque, P. (1990). An antibiotic assay by the agar-well diffusion method. Acta Biol. Med. Exp. 15: 113-115.
20. Pitout, J. (2008) Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis.* 8(3): 159–166.
21. Sarika R.,Sonalika M.,Hridya C.and V. Srikanth. (2021). Molecular determinants of peaceful coexistence versus invasiveness of non-Typhoidal Salmonella: Implications in long-term side-effects.Molecular Aspects of Medicine. Volume 81, October 2021, 100997.
22. Scalbert, A. (1991): Antimicrobial properties of tannins. **Phytochemistry**30: 3875–3883
23. Scher, A. (2009). Antimicrobial activity of natural products from medicinal plants. *Gomal Journal of medicinal science,* 7: 72-78.
24. Simlai A. & Roy A. (2012). Analysis of and correlation between phytochemical and antimicrobial constituents of Ceriops decandra, a medicinal mangrove plant, from Indian Sundarban estuary.J. Med. Plants Res., 6 (32), pp. 4755-4765.
25. Trease, G. E. and Evans, W. C. (2008). Pharmacognosy. Bailliere Tindall, London. 223-389
26. WHO (2019). WHO global report on traditional and complementary medicine. World
27. Newman, D.J., Cragg, G.M. and Snader, K.M. (2003). Natural products as sources of new drugs over the period 1981–2002. Journal of Natural Products 66(7): 1022–1037.
28. Perez, C., Pauli, M. and Bazerque, P. (1990). An antibiotic assay by the agar-well diffusion method. Acta Biol. Med. Exp. 15: 113-115.