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RESEARCH ARTICLE

POTENT ANTIBACTERIAL EFFECTS OF *TERMINALIA SUPERBA* ENGL. AND DIELS (COMBRETACEAE) BARK EXTRACTS

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Abstract

Antibiotic misuse has caused widespread multi-resistance in Enterobacteriaceae. Our study investigates *Terminalia superba* (*T. superba*) extracts against Extended-spectrum beta-lactamases (ESBL)-producing strains. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using broth microdilution (Mueller-Hinton, 10^6 CFU/mL). The extract concentrations ranged from 0.078 to 80 mg/mL. We evaluated *T. superba* extract's β-lactamase inhibition of nitrocefin hydrolysis, estimated inhibition percentages, and IC_{50} values for each extract. The double-disk test confirmed the presence of ESBLs in the strains, as all of them exhibited hydrolytic activity. The results indicated that the *T. superba* extracts displayed dose-dependent inhibitory effects on beta-lactamase. Among them, the hydro-ethanolic extract displayed potent inhibitory activity against β-lactamases produced by ESBL-E. coli (IC_{50} = 0.065 mg/mL), ESBL-K. pneumoniae (IC_{50} = 0.076 mg/mL), and ESBL-P. mirabilis (IC_{50} = 0.082 mg/mL). However, following the removal of tannins, the hydro-ethanolic extract's anti-β-lactamase activity was reduced. Our findings highlight the remarkable in vitro activity of *T. superba* extracts against ESBL-producing Enterobacteriaceae, suggesting their potential clinical utility in addressing infections caused by these drug-resistant pathogens. The exploration of *T. superba* extracts as a possible source of effective anti-ESBL agents could contribute significantly to combating antibiotic resistance.

Introduction:-

Among the broad classes of antibiotics are the β-lactam antibiotics. This class includes well-known antibiotics such as penicillin, cephalosporin, monobactam, and carbapenem. In response to the growing number of multi-resistant bacterial strains, especially those bacteria that produce extended-spectrum β-lactamases (ESBLs), β-lactamases have gained increasing prominence as enzymes that confer resistance against β-lactam antibiotics(Jones et al., 2004). The primary mechanism responsible for microbial resistance to β-lactam antibiotics is hydrolysis by these β-lactamases(Bradford, 2001). Gram-negative bacteria are known to produce various types of β-lactamases, each with

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a different substrate profile. The significance of β-lactamases in the bacterial response to specific antibiotics lies in their capacity to either complement the basic tolerance or compensate for its lack (Cherkaoui et al., 2004). As current antibiotic therapy options become increasingly limited, there is a pressing need to discover new antimicrobial agents capable of combating multi-drug resistant bacteria. Standardized extracts from plants present a promising source of novel and secure antibacterial medications due to the diverse chemical compounds they contain (Newman and Cragg, 2012).

Plants are known to produce a wide array of chemical compounds with the potential to inhibit pathogens. Thus, the screening of herbal extracts becomes vital to identify β-lactamase inhibitors that can potentially work synergistically with existing antibiotics to impede the development of antibiotic resistance. In this context, the focus of the present study was on *Terminalia superba* Engl. and Diels (Combretaceae). Traditional healers have long utilized *T. superba* to address infections caused by bacteria, fungi, and viruses. The objective of this research was to ascertain in vitro the activity of *T. superba* extracts counter to ESBL-producing clinical isolates, aiming to explore their potential as effective agents against multi-resistant bacterial strains.

Materiel and Methods:-

Plant material

The barks of *T. superba* were harvested in Itchèdé, Toffo Forest, Adja-Ouèrè, Republic of Benin. The plant has been identified and verified at the National Herbarium in the University of Abomey-Calavi. The gathered barks were air-dried at 25°C and finely milled into a powder.

Microorganisms

The efficacy of *T. superba* extracts in inhibiting the growth of various bacterial strains was evaluated, including *Escherichia coli* ATCC 25922 (β-lactamase negative), *Klebsiellapneumoniae* ATCC 700603 (Extended-spectrum beta-lactamase (ESBL) positive), and enterobacteria multidrug-resistant that produce extended-spectrum β-lactamase (ESβL) (*Escherichia coli*, *Klebsiellapneumoniae*, *Proteus mirabilis*)**.** These strains were grown in Muller-Hinton broth with 20% glycerol and stored at -80˚C.

Preparation of extracts

The extracts were generated with the approach outlined by Talbi et al. (Talbi et al., 2015). 50 grams of *T. superba* powder were extracted in 100% ethanol (ethanolic extract) and 70% ethanol (hydro-ethanolic extract). The obtained extract was dried using aRotavapor.

Estimation of Phenolic, Flavonoid, Tannin contents

Total phenolic content of different extracts of *T. superba* was determined employing the Folin-Ciocalteu technique (Singleton and Rossi, 1965).The flavonoid content was determined by the aluminum trichloride method(Zhishen et al., 1999).Total tannin content was measured via UV-Vis spectrophotometry at a maximum wavelength of 745 nm using tannic acid as a standard(Gurning et al., 2021).

Antimicrobial susceptibility testing

Testing for antibiotic susceptibility was carried out using the disk diffusion technique (Kirby Bauer) on *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*(Clinical and Laboratory Standards Institute (CLSI), 2008). Various antibiotics were tested, including Beta-lactams: Amoxicillin (20µg), Amoxicillin plus clavulanic acid (20-10µg), Imipenem (10µg), Cefotaxime (5µg), Ceftazidime (10µg), Ceftriaxon (30µg), Cefazolin (30µg), Cefoxitin (30µg), Aztreonam (30µg) ; Aminoglycosides: Gentamicin (10µg), Amikacin (30µg), Netilmicin (10µg) ; Phenicolated: Chloramphenicol (30µg) ; Quinolone and Fluoro-Quinolones: Nalidixic acid (30µg), Ciprofloxacin(5µg), Pefloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg); Cyclines:Doxycycline (30µg), Tetracycline(30µg), Nitrofurans: nitroxoline (30µg); Polypeptides: Colistin (50µg); Sulfonamides and associates: Trimethoprim plus Sulfamethoxazole (1,25-23,75 µg).

Identification of Extended-Spectrum Beta-Lactamase

To confirm ESBL production in *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis*, the double disc diffusion synergy test (DDST) was used(Kaur et al., 2013).Various beta-lactam discs (amoxicillin (20µg), imipenem(10µg), cefotaxim (5µg), ceftazidim (10µg), ceftriaxon (30µg), cefazolin (30µg), cefoxitin (30µg), aztreonam (30µg)) were placed near (20 to 30 mm) the amoxicillin plus clavulanic acid (20-10 µg) disc and ESBL production was determined based on the increase in zone size towards the clavulanic acid disc.

Assessment of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of *T. superba* stem bark extracts

T. superba extracts were reconstituted in distilled water at a concentration of 80 mg/mL and sterilized through filtration. The antibacterial activity was assessed using a microwell dilution technique, and the MIC and MBC values were obtained. The extracts were classified as bactericidal or bacteriostatic based on their MBC/MIC ratios(Elisha et al., 2017).

Effects of *Terminalia superba* against β-lactamase producingstrains

Preparation of crude beta-lactamase enzyme

Crude beta-lactamase enzyme was prepared from clinical strains of *Escherichia coli*, *Klebsiellapneumoniae*, or *Proteus mirabilis*. The cells were harvested, washed, and disrupted using an ultrasonic disintegrator. The enzymatic activity was computed at 630 nm and stored at -20°C. ESBL production was confirmed by the hydrolysis of β-lactam antibiotics(Martínez-Martínez et al., 1996).

Elimination of tannins

The crude hydro-ethanolic extracts were treated with hide powder to remove tannins according to the European Pharmacopeia method. The resulting solution was lyophilized and dissolved in sodium phosphate buffer for enzyme activity tests(Livermore and Woodford, 2006).

Beta-latamase inhibition assay

Hydro-ethanolic extract of *T. superba* and hydro-ethanolic extract of *T. superba* after elimination of tannins underwent testing for their capability to inhibit the hydrolysis of nitrocefin by β-lactamase. Clavulanic acid was used as a control. The percentage inhibition of β-lactamase was computed. IC_{50} values were established for each extract.

Statistical analysis

Data were analyzed using Excel and SPSS 17, with calibration curves created in Excel. We calculated means, standard deviations, and conducted Student's Levene and t-tests for variance and mean comparisons, respectively. Non-parametric tests were used for non-normally distributed variables, and significance was set at $p < 0.05$.

Results:-

Mesurement ofphenolic, flavonoid and tannin content

The levels of total phenolic (304.33 ± 4.04 vs. 283.67 ± 3.21 mg gallic acid equivalents per gram of extract; $p<0.002$), total flavonoid (30 ± 1.58 vs. 28 ± 1.00 mg quercetin equivalents per gram of extract; $p<0.004$), and total tannin content (211.00 ± 3.61 vs. 197.67 ± 2.52 mg tannic acid equivalents per gram of extract; $p<0.006$) were significantly higher in hydro-ethanolic extract compared to the ethanol extract of *T. superba* bark (Table 1).

Table 1:-Phenolic, flavonoïd and tannin content in ethanol and hydroethanol extracts of T. superb.

	Total Phenol (mg AGE/g)	Total Flavonoid (mg QE/g)	Total Tannin (mg ATE/g)
Ethanolic extract	283.67 ± 3.21	28 ± 1.00	197.67 ± 2.52
Hydroethanolic extracts	304.33 ± 4.04	30 ± 1.58	211.00 ± 3.61
P. value	0.002	0.044	0.006
Calibration curve equation	y = 0.213x – 0.004 R^2 = 0.991	y = 15.68x + 0.007 R^2 = 0.998	y = 0.296 x + 0.001 R^2 = 0.991

mg EAG/g : mg gallic acid equivalents/g dry weight of extract; mg EQ/g : mg quercetin equivalents/g dry weight of extract; mgAT/g : mg tannic acid equivalents/g dry weight of extract.

Confirmation of multidrug-resistant extended-spectrum beta-lactamases (ESBLS)-producing enterobacteriaceae strain

Results of the antibiotic sensitivity test (Table 2) revealed that all strains demonstrated resistance to Beta-lactams (Amoxicillin, Cefotaxime, Ceftazidime, Ceftriaxone, Cefazolin, Cefoxitin, Aztreonam) but remained susceptible to amoxicillin plus clavulanic acid and imipenem. All strains were found to be susceptible to nitrofuranes (nitroxoline), polypeptides (colistin), sulfamides, and combinations thereof (trimethoprim plus sulfamethoxazole). *Escherichia coli* exhibited susceptibility to aminoglycosides (Amikacin, Netilmicin) and quinolones (nalidixic acid, Ciprofloxacin, Ofloxacin). *K. pneumoniae* demonstrated susceptibility to aminoglycosides (Amikacin) and quinolones (nalidixicacid). *Proteus mirabilis*, on the other hand, was susceptible to quinolones (Ofloxacin,

Norfloxacin). The double disk synergy test confirmed the presence of extended-spectrum beta-lactamases (ESBLs) in the strains under investigation.

Table 2:- Antimicrobialsusceptibility.

Antibiotics		*Escherichia coli*	*Klebsiellapneumonia*	*Proteus mirabilis*
Beta-lactams	Amoxicillin (20µg)	R	R	R
	Amoxicillin + clavulanic acid (20-10 µg)	S	S	S
	Imipenem (10µg)	S	S	S
	Cefotaxim (5µg)	R	R	R
	Ceftazidim (10µg)	R	R	R
	Ceftriaxon (30µg)	R	R	R
	Cefazolin (30µg)	R	R	R
	Cefoxitin (30µg)	R	R	R
	Aztreonam 30µg)	R	R	R
Aminoglycosides	Gentamicin (10µg)	R	R	R
	Amikacin (30µg)	S	S	R
	Netilmicin (10µg)	S	R	R
Phenicols	Chloramphenicol (30µg)	R	R	R
Quinolone and Fluoro-Quinolones	Nalidixic acid (30µg)	S	S	R
	Ciprofloxacin (5µg)	S	R	R
	Pefloxacin (5µg)	R	R	R
	Levofloxacin (5µg)	R	R	R
	Ofloxacin (5µg)	S	R	S
	Norfloxacin (10µg)	R	R	S
Cyclines	Doxycycline (30µg)	R	R	R
	Tetracycline (30µg)	R	R	R
Nitrofurans	nitroxoline (30µg)	S	S	S
Polypeptides	Colistin (50µg)	S	S	S
Sulfonamides and associates	Trimethoprim + Sulfamethoxazole (1.25-23.75 µg)	S	S	S

R : Resistant ; S : susceptible

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Finding indicated that MIC and MIB varied according to the bacterial strains. The MIC values varied from 0.078 to 0.312 mg/mL for both extracts on reference strains. On isolated strains, MIC values varied from 0.312 to 0.625 mg/mL for the ethanolic extract and 0.156 to 0.312 mg/L for the hydro-ethanolic extract. The MBC varied from 0.078 to 0.625 mg/mL for the extracts on reference strains. On isolated strains, MBC values varied from 0.625 to 2.5 mg/mL for ethanolic extracts and varied from 0.312 to 0.625 mg/mL for hydro-ethanolic extracts. Both the ethanolic extract and the hydro-ethanolic extract exhibit bactericidal effects on all strains.

Table 3:- Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of *T. superba*extracts on reference strains and isolated strains.

	Ethanolic extract			Hydro-ethanolic extract		
	MIC (mg/mL)	MBC (mg/mL)	$\frac{MBC}{MIC}$	CMI (mg/mL)	CMB (mg/mL)	$\frac{MBC}{MIC}$
Escherichia coli ATCC 25922	0.156	0.312	2	0.156	0.156	1
*Klebsiella pneumoniae*ATCC 700603	0.312	0.625	2	0.312	0.625	2
E. Coli ESBL+	0.312	0.625	2	0.156	0.312	2
K. Pneumoniae ESBL+	0.625	1.25	2	0.312	0.625	1

P. Mirabilis ESBL +	0.625	2.5	4	0.312	0.625	2

E. Coli ESBL+ : *Escherichia coli*,which generate extended- spectrum beta-lactamases (ESBLs) ; *K. Pneumoniae* ESBL+ : *Klebsiella pneumoniae*, which generate extended- spectrum beta-lactamases (ESBLs); *P. Mirabilis* ESBL + : *Proteus mirabilis*,which generate extended- spectrum beta-lactamases (ESBLs); MIC : Minimal Inhibitory Concentration (mg/ml); MBC : Minimal Bactericidal Concentration (mg/ml); CMB/CMI : antibiotic power

***Terminalia superba* effect against extended spectrum beta-lactamase enzymes produced by strains**

The ability of hydro-ethanolic extracts (HEE) and the hydro-ethanolic extracts after elimination of tannin (HEEdT) to inhibit the activity of ESBL enzymes was tested in vitro using nitrocefin as a chromogenic substrate. Clavulanic acid was employed as a control. The treatment with *T. superba* extracts displayed a dose-dependent reduction in the activity of ESBL enzymes produced by *Escherichia coli*, *Klebsiellapneumoniae*, and *Proteus mirabilis*.

Table 4 presents the IC_{50} values for *T. superba* extracts and reference standard, with lower IC_{50} values indicating higher radical scavenging activity. The IC_{50} of the hydro-ethanolic extract (0.065 mg/mL) is 2.06 times lower than that of the hydro-ethanolic extract after tannin removal (0.134 mg/mL) and 27.08 times higher than that of clavulanic acid (0.0024 mg/ml).

The IC_{50} of the hydro-ethanolic extract (0.076 mg/mL) is 2.18 times lower than that of the hydro-ethanolic extract after tannin elimination (0.166 mg/mL) and 20 times higher than that of clavulanic acid (0.0038 mg/mL).

The IC_{50} of the hydro-ethanolic extract (0.082 mg/mL) is 2.57 times lower than that of the hydro-ethanolic extract after tannin removal (0.211 mg/mL) and 18.63 times higher than that of clavulanic acid (0.0044 mg/mL).

The hydro-ethanolic extract of *T. superba* bark demonstrates a greater capacity to inhibit extended-spectrum beta-lactamases (ESBLs) compared to the extract after tannin elimination. However, the ESBL inhibitor activity of the hydro-ethanolic extract is lower than that of clavulanic acid.

Table 4:-IC_{50} of extracts (hydro-ethanolic and after elimination of tannin) of *T. superba* barks.

		Hydro-ethanolic	Hydro-ethanolic after elimination of tannin	Clavulanic acid
ESBL-E. Coli	IC_{50}	0.065 mg/mL	0.134 mg/mL	0.0024 mg/mL
	Calibration curve equation	Y= 26.53ln(x) + 122.28 R^2 = 0.8875	Y= 33.596ln(x) + 117.42 R^2 = 0.9316	Y= 9.9483ln(x) +109.68 R^2 = 0.7948
ESBL-*K.pneumoniae*	IC_{50}	0.076 mg/mL	0.166 mg/mL	0.0038 mg/mL
	Calibration curve equation	Y= 28.405ln(x) + 123.16 R^2 = 0.8913	Y= 36.914ln(x) + 116.17 R^2 = 0.9482	Y= 10.957ln(x) + 110.95 R^2 = 0.7984
ESBL-P. mirabilis	IC_{50}	0.082 mg/mL	0.211 mg/mL	0.0044 mg/mL
	Calibration curve equation	Y= 28.982ln(x) + 122.44 R^2 = 0.9026	Y= 31.005ln(x) + 98.194 R^2 = 0.9701	Y= 11.39ln(x) + 111.56 R^2 = 0.7905

ESBL-E. Coli: extended- spectrum beta-lactamases (ESBLs) producingEscherichia coli; ESBL-*K.pneumoniae*: extended- spectrum beta-lactamases (ESBLs) producing*Klebsiellapneumoniae*; ESBL-P.mirabilis:extended-spectrum beta-lactamases (ESBLs) producing *Proteus mirabilis*

Discussion:-

Emerging multidrug-resistant bacteria pose significant health risks to both humans and animals. β-Lactamases, which are generated by a range of bacteria, are enzymes that render β-Lactam antibiotics ineffective by breaking open their β-Lactam ring structure(Jones et al., 2004). Roughly 60% of all antibacterial agents employed in the treatment of infections caused by Gram-negative bacteria consist of β-Lactam antibiotics (Livermore and Woodford, 2006).Bacteria mainly develop resistance against β-lactam antibiotics through the synthesis and secretion of β-Lactamase, resulting in the destruction of the antibiotic's β-lactam ring. As antibiotic resistance among human pathogens continues to increase rapidly, there is a pressing need to actively seek new inhibitors for beta-lactamase enzymes(Madhavan and Murali, 2011).

In the fight against antibiotic resistance in disease-causing bacteria, the utilization of medicinal plants has been advocated due to the diverse range of compounds present in herbal formulations (Rubens et al., 2015). Consequently, there is an urgent requirement to develop effective drug therapies.Results confirmed that the strains of Proteus mirabilis, *Escherichia coli* and *Klebsiella pneumoniae* used in this research were multidrug resistant bacteria that produce extended spectrum β-lactamases (ESBLs) as assessed through antimicrobial susceptibility testing, the double disk synergy test, and antibiotic hydrolysis. Our findings demonstrate that both the ethanol and 70% hydro-ethanol extracts of *T. superba* storm bark effectively exhibit antimicrobial activities against bacteria. Previous research has also reported the antibacterial effectiveness of extracts from *T. superba* against various multi-drug resistant (MDR) microorganisms(Ahon et al., 2011; Kougnimon et al., 2018).

The analysis of phytochemical compounds of plant extracts suggests the presence of different groups of phytoconstituents, including flavonoids, phenols, glycosides,, tannins, saponin, etc.,which can be responsible for the antibacterial activity individually or in combination(Ahmad and Aqil, 2007).*T. superba* contains secondary metabolites, including tannins, flavonoids, saponosides, reducing compounds, free anthracenics, and mucilage(Ahon et al., 2011; Kougnimon et al., 2018)which exert antibacterial activity through various mechanisms, such as damaging the bacterial membrane, suppressing virulence factors, inhibiting the activity of enzymes and toxins, and preventing bacterial biofilm formation(Barbieri et al., 2017; Khameneh et al., 2019).The hydro-ethanolic extract was selected for further investigation based on its antimicrobial activity. Once tannins were removed from the hydro-ethanolic extract, further examinations were conducted to assess their anti-β-lactamase properties.

The inhibitory activity of the extracts varied significantly in the presence of different β-lactamases produced by bacteria. *T. superba* bark hydro-ethanolic extract exhibited a higher capacity to inhibit ESBLs compared to the extract after tannin elimination, indicating that tannins play a role in this phenomenon. Tannins possess antibacterial efficacy by penetrating the bacterial cell membrane, interfering with the cell's metabolism, and causing its destruction. Tannic acid, for example, can inhibit bacterial attachment to surfaces, resulting in the death of bacteria cells unable to adhere to the surface. Additionally, tannic acid hinders the uptake of sugar and amino acids, thereby restricting bacterial growth. However, the impact of phenolic acid on bacteria is dependent on various factors such as concentration, pH, temperature, and the type of matrices in which tannic acid is incorporated. These compounds have also been reported to have potential antiviral, antibacterial, antiparasitic, and anticancer effects(Kaczmarek, 2020).

Furthermore, our results revealed that, in addition to tannins, other compounds in the extract also exhibit inhibitory activity on extended-spectrum β-lactamases (ESBLs). Previous studies have indicated a relation between the inhibitor activity of ESBLs and the phenolic content(Fu et al., 2016; Saeidi et al., 2015).

Conclusion:-

We showed in this study that *T. superba* contain bioactive compounds that inhibit beta-lactamases. The hydro-ethanolic extract presented the highest enzyme inhibition activity among the tested extracts, suggesting its potential as a source for developing beta-lactamase inhibitors. Results highlight the significance of exploring natural products like *T. superba* for developing effective therapeutic agents against drug-resistant bacterial infections.

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References:-

1. Ahmad, I., and Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research, 162(3), 264–275. https://doi.org/10.1016/j.micres.2006.06.010
2. Ahon, M., Akapo-Akue, J., and Kra, M. (2011). Antifungal activity of the aqueous and hydro-alcoholic extracts of *Terminalia superba* Engl. On the in vitro growth of clinical isolates of pathogenic fungi. Agriculture and Biology Journal of North America, 2(2), 250–257.

3. Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., and Nabavi, S. M. (2017b). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. Microbiological Research, 196, 44–68. https://doi.org/10.1016/j.micres.2016.12.003
4. Bradford, P. A. (2001). Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Reviews, 14(4), 933–951, table of contents. https://doi.org/10.1128/CMR.14.4.933-951.2001
5. Cherkaoui, A., Emonet, S., Renzi, G., Riat, A., Greub, G., and Schrenzel, J. (2004). Bêtalactamases à spectre étendu et carbapénémases chez les Enterobacteriaceae. Revue Médicale Suisse, 10, 2142–2148.
6. Clinical and Laboratory Standards Institute (CLSI). (2008). Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Informational Supplement (Document M100-S18), The Clinical and Laboratory Standards Institute, Wayne, 4, 354.
7. Elisha, I. L., Jambalang, A. R., Botha, F. S., Buys, E. M., McGaw, L. J., and Eloff, J. N. (2017). Potency and selectivity indices of acetone leaf extracts of nine selected South African trees against six opportunistic Enterobacteriaceae isolates from commercial chicken eggs. BMC Complementary and Alternative Medicine, 17(1), 90. https://doi.org/10.1186/s12906-017-1597-3
8. Fu, L., Lu, W., and Zhou, X. (2016). Phenolic Compounds and In Vitro Antibacterial and Antioxidant Activities of Three Tropic Fruits: Persimmon, Guava, and Sweetsop. BioMed Research International, 2016, 1–9. https://doi.org/10.1155/2016/4287461
9. Gurning, K., Simanjuntak, H. A., Purba, H., Situmorang, R. F. R., Barus, L., and Silaban, S. (2021). Determination of Total Tannins and Antibacterial Activities Ethanol Extraction Seri (Muntingia calabura L.) Leaves. Journal of Physics: Conference Series, 1811(1), 012121. https://doi.org/10.1088/1742-6596/1811/1/012121
10. Jones, M. E., Draghi, D. C., Thornsberry, C., Karlowsky, J. A., Sahm, D. F., and Wenzel, R. P. (2004). Emerging resistance among bacterial pathogens in the intensive care unit—A European and North American Surveillance study (2000-2002). Annals of Clinical Microbiology and Antimicrobials, 3, 14. https://doi.org/10.1186/1476-0711-3-14
11. Kaczmarek, B. (2020). Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials—A Minireview. Materials, 13(14), 3224. https://doi.org/10.3390/ma13143224
12. Kaur, J., Chopra, S., Sheevani, null, and Mahajan, G. (2013). Modified Double Disc Synergy Test to Detect ESBL Production in Urinary Isolates of *Escherichia coli* and *Klebsiella pneumoniae*. Journal of Clinical and Diagnostic Research: JCDR, 7(2), 229–233. https://doi.org/10.7860/JCDR/2013/4619.2734
13. Khameneh, B., Iranshahy, M., Soheili, V., and Fazly Bazzaz, B. S. (2019). Review on plant antimicrobials: A mechanistic viewpoint. Antimicrobial Resistance and Infection Control, 8(1), 118. https://doi.org/10.1186/s13756-019-0559-6
14. Kong, K.-F., Schneper, L., and Mathee, K. (2010). Beta-lactam antibiotics: From antibiosis to resistance and bacteriology. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica, 118(1), 1–36. https://doi.org/10.1111/j.1600-0463.2009.02563.x
15. Kougnimon, F. E. E., Akpovi, C. D., Dah Nouvlessounon, D., Boya, B., Baba Moussa, L., and Loko, F. (2018). Antioxidant and Antibacterial Activities of *Terminalia superba* Engl. And Diels (Combretaceae) Bark Extracts. International Journal of Current Microbiology and Applied Sciences, 7(07), 2836–2846. https://doi.org/10.20546/ijcmas.2018.707.332
16. Livermore, D. M., and Woodford, N. (2006). The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends in Microbiology, 14(9), 413–420. https://doi.org/10.1016/j.tim.2006.07.008
17. Madhavan, H., and Murali, S. (2011). Mechanisms of development of antibiotic resistance in bacteria among clinical specimens. Journal of Clinical and Biomedical Sciences, 1(2), 42–48.
18. Martínez-Martínez, L., Hernández-Allés, S., Albertí, S., Tomás, J. M., Benedi, V. J., and Jacoby, G. A. (1996). In vivo selection of porin-deficient mutants of Klebsiella pneumoniae with increased resistance to cefoxitin and expanded-spectrum-cephalosporins. Antimicrobial Agents and Chemotherapy, 40(2), 342–348. https://doi.org/10.1128/AAC.40.2.342
19. National Committee for Clinical Laboratory Standards (NCCLS). (2001). Performance standards for antimicrobial susceptibility testing; 11th informational supplement.
20. Newman, D. J., and Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products, 75(3), 311–335. https://doi.org/10.1021/np200906s
21. Rubens, D., Constantin, O., and Moevi, A. (2015). Staphylococcus aureus activity of the aqueous extract and hexanic fraction of Thonningia sanguinea (Cote ivoire). International Journal of Pharmacognosy and Phytochemical Research, 7(2), 301–306.

22. Saeidi, S., Amini Boroujeni, N., Ahmadi, H., and Hassanshahian, M. (2015). Antibacterial Activity of Some Plant Extracts Against Extended- Spectrum Beta-Lactamase Producing *Escherichia coli* Isolates. Jundishapur Journal of Microbiology, 8(2), e15434. https://doi.org/10.5812/jjm.15434
23. Singleton, V., and Rossi, J. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic, 16, 44–158.
24. Talbi, H., Boumaza, A., El-mostafa, K., Talbi, J., and Hilali, A. (2015). Evaluation de l'activité antioxydante et la composition physico-chimique des extraits méthanolique et aqueux de la NigellasativaL. Journal of Materials and Environmental Science, 6(4), 1111–1117.
25. Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4), 555–559.