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Phytochemical Analysis and Antibacterial Activity of Methanol and Ethyl Acetate Extracts of *Detarium microcarpum* Guill. & Perr.

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Abstract

This research aimed to investigate the phytoconstituents and antibacterial effects of methanol and ethyl acetate stem bark extracts of *Detarium microcarpum* (DM). The phytochemicals were detected and quantified while the antibacterial activity *against Escherichia coli, Staphylococcus aureus, and Salmonella typhi* was established determining the zone of inhibition (ZI), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). Phytochemical screening showed alkaloids (16.33% ±0.88) were present in the methanol extract only while saponins and flavonoids were detected in concentrations of 31.00% ±2.31 and 21.01% ±2.33 respectively for the methanol extract and 21.67% ±1.76 and 38.01% ±1.16, for the ethyl acetate. The methanol extract exhibited the highest ZI on *S. aureus* (21.3 mm ±1.11) with its least inhibition observed on *E. coli* (6.5 mm ±0.77) while the ethyl acetate extract demonstrated the highest ZI on *S. typhi* (19.1 mm ±2.01). *S. typhi* exhibited more sensitivity to DM extracts at the least concentrations of 12.5 mg/ml (methanol) and 25 mg/ml (ethyl acetate) while the MBC results showed that the 12.5 mg/ml and 25 mg/ml were the effective respective concentration for methanol and ethyl acetate extracts against *S. typhi*. Conclusively, DM exhibited an antibacterial effect against the test organisms with notable inhibitory and bactericidal effects.

Keywords: Antibacterial activity; Antimicrobial activity; Antibacterial resistance; *Detarium microcarpum*; Phytochemical analysis.

Abbreviations: DM (*Detarium microcarpum*), ESBE (Ethyl acetate stem bark extract), MSBE (Methanol stem bark extract), MIC (Minimum inhibitory concentration), MBC (Minimum bactericidal concentration), and ZI (Zone of inhibition).

INTRODUCTION

Medicinal plants have long been reported to be sources of therapeutics and have been utilized in the management of several ailments in traditional and folkloric practices. Different research reported the pharmacological effects of several plants employed to manage ailments including diabetes, cancer, inflammation, parasite, and microbial infections (Prasathkumar *et al.*, 2021). In traditional practice, medicinal plant parts such as roots, leaves, and stem bark are prepared in different forms such as decoction and infusions administered by oral or inhalation as an alternative to modern medicines to achieve therapeutic purposes (Ullah *et al.*, 2020). There are several reasons for the inclination toward the use of medicinal plants as it offers certain advantages such as being cheap, and available with minimized side effects compared to modern drugs (Ahad *et al.*, 2021; Rahayu *et al.*, 2020). In other cases, poverty, poor health facilities, and access may contribute to the recognition of drugs from plant sources as alternatives. The therapeutic use of plants as sources of drugs in disease and infection management has been attributed to their phytochemical components extracted via the preparation method which are associated with several biological effects (George *et al.*, 2021; Hossen *et al.*, 2022; Zheng *et al.*, 2022).

Bacterial infections are attributed as the cause of millions of deaths and morbidities worldwide and have so far evolved into a major health concern all over the world (Ji *et al.*, 2016). Although different antibiotics of varying efficacy are employed in the treatment of infections, these antibiotics are losing their efficacy because of antimicrobial resistance which is a major challenge in achieving treatment goals (Maillard *et al.*, 2020). Antimicrobial resistance forms a major problem in treatment of infectious diseases especially in rural communities and developing countries even though this resistance continues to occur every day during treatment with an estimated projected death of up to 4 million by 2050 (Akinde & Taiwo, 2017; Elton *et al.*, 2020; Tong *et al.*, 2019). There is an increase in antimicrobial resistance, especially in Africa, however, phytochemicals from plants serve as long-time sources of therapeutics with proven results in treatments of infectious diseases

(Bouyahya *et al.*, 2017). Phytochemicals from plants offer an alternative as utilized in traditional practices as infectious diseases are a worldwide menace and create a burden on an individual and governments.

Phytochemicals extracted from different plant preparations exert different pharmacological effects individually or synergistically observed in the folkloric use of plant drugs. Alkaloids are associated with anti-diabetic (Adhikari, 2021), anticancer (Song *et al.*, 2022), and antimicrobial effects (Fan *et al.*, 2022; Jafaar *et al.*, 2021). Flavonoids exert biological and pharmacological activities against oxidative stress, cancer, inflammation, and tumor through different modes of action (Jucá *et al.*, 2020). Flavonoids also exert an antimicrobial effect on antibiotic-resistant microbes (Biharee *et al.*, 2020; Gupta *et al.*, 2022). Glycosides exhibit pharmacological effects including anti-diabetic (Yang *et al.*, 2022) and antimicrobial activities (Tm *et al.*, 2022). Terpenoids exert pharmacological effects against a tumor, cancer, inflammation, malaria, diabetes, and microbial infections (Wu *et al.*, 2020; Yang *et al.*, 2020). Saponins exhibit pharmacological effects against cancer, oxidative stress, inflammation, and microbial infections (Fang *et al.*, 2020). Several phytochemicals exert different pharmacological activities working either individually or in synergy with each other. Thus, this research aimed to establish the phytochemical composition and antibacterial effects of methanol and ethylacetate extracts of DM.

MATERIALS AND METHODS

Study area

Detarium microcarpum (DM) sample collection was carried out in the Girei Local Government Area of Adamawa state, Nigeria. The authentication of the plant sample was done by a Forest Technologist from the Department of Forestry, Adamawa State Polytechnic Yola, where a voucher specimen (ASP/FT/0212) was deposited. The stem bark was dried and ground into powder using a blender.

Chemicals and reagents

All the chemicals and reagents used for the study were of AnarlaR.

Procedures

Extract preparation

Stem bark powder (300 g) of DM was macerated in 1 L of 70% v/v methanol and ethyl acetate for 48 hours, followed by filtration and concentrated to dryness over reduced pressure (Evans, 2009).

Qualitative phytochemical Analysis

The detection of phytochemicals in methanol (MSBE) and ethyl acetate (ESBE) stem bark extracts of DM was carried out following the methods previously described (Evans, 2009).

Quantitative phytochemical analysis

- *Alkaloids*
  Alkaloids were quantified as previously described (Harborne, 1998).
- *Saponins*
  Saponins were estimated by the method previously described (Obadoni & Ochuko, 2002).
- *Flavonoid*
  Quantitation of flavonoids was carried out by the method previously (Harborne, 1998).

Antibacterial activity

- *Test organisms*
  The bacteria isolates (*E. coli, S. aureus, and S Typhi*) were collected from Modibbo Adama University Teaching Hospital microbiology laboratory, Yola, Nigeria. The characterization of the test organisms was done through observation of their cultural growth characteristics according to a previously described method (Idris Abubakar & Abubakar Usman, 2016). Confirmation of clinical isolates' identity was carried out *via* biochemical tests following the standard method previously described (Cheesbrough, 2002; Talaiekhozani, 2013). The pure cultures were streaked onto a nutrient agar slant followed by 24 h incubation at 37 °C and subsequent storage at 4 °C until needed for use.
- *McFarland Standard Preparation*
  Exactly 9.95 mL of 1% H_2SO_4 and 0.05 mL of 1.17% BaCl were mixed to form a precipitated suspension acting as 0.5 McFarland standard set as the turbidity for the test organisms (Cheesbrough, 2002).
- *Inoculum Standardization*
  The confirmed isolates were subjected to further culturing on nutrient agar containing petri-dishes followed by overnight incubation at 37 °C to form colonies which were transferred into test-tubes / containing 5 mL of 0.9% normal saline adjusted to the turbidity of previously prepared McFarland's standard (Andrews, 2005).

Determination of antibacterial Activity

A slightly modified agar well diffusion technique was adopted to establish the antibacterial effects of DM (Biradar *et al.*, 2008). The bacterial inoculation was carried out with a sterile swab onto a prepared solidified Mueller-Hinton agar, and allowed to stand for 15 min, followed by the addition of 0.2 mL of the extracts of varied concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) to five wells bored with cork borer with the addition of Ciprofloxacin to the other well as a positive control and incubated overnight at 37°C. The diameter of the zones of

inhibition was used to determine the antibacterial effects of the extracts.

Determination of Minimum Inhibitory Concentration (MIC)

The previously described method was adopted to ascertain the MIC of DM following the procedures described by National Committee for Clinical Laboratory Standards (NCCLS) (Lar *et al.*, 2011). Briefly, one mL of concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL) of the extract was introduced into seven tubes with 5 mL of Muller-Hinton broth and thoroughly mixed, then 0.1 mL of broth cultures of the test organism and overnight incubation at 37 °C for bacterial growth to be observed. The minimum extract concentration to inhibit bacterial growth was defined as the MIC of the extracts.

Determination of Minimum Bactericidal Concentration (MBC)

The evaluation of the MBC was carried out by further culturing the test-tubes with no visible growth in the MIC test on Mueller-Hinton agar by spreading 0.1 mL of the inoculum with a sterile loop, followed by overnight incubation at 37 °C. The minimum concentration with no visible growth was defined as the MCB (De & Ifeoma, 2002).

Data analysis

Data obtained were expressed as mean ± standard error of triplicate determinations' mean (± SEM) and evaluated with Statistical Package for the Social Sciences (SPSS) version 22 Software.

RESULTS AND DISCUSSION

The phytochemicals detected in the MSBE and ESBE of DM are presented in Table 1. Alkaloids were detected in only the MSBE while saponins and flavonoids were detected in both the MSBE and ESBE. However, steroids, glycosides, and terpenoids were absent in both the MSBE and ESBE.

Table 1. Qualitative phytochemical test of MSBE and ESBE of DM.

Phytochemicals	Inference	
	MSBE	ESBE
Alkaloids	+	-
Saponins	+	+
Steroids	-	-
Glycosides	-	-
Terpenoids	-	-
Flavonoids	+	+

Note: + = present, - = absent.

The phytochemicals quantified in the MSBE and ESBE are shown in Table 2. Alkaloids were quantified up to 16.33 % ±0.88 in the MSBE while saponins quantified up to 31.00 % ±2.31 and 21.67 % ±1.76 for MSBE and ESBE respectively. The concentration of flavonoids in the MSBE was 21.01 % ±2.33 while that of the ESBE was 38.01 % ±1.16.

Table 2. Quantitative phytochemical composition of MSBE and ESBE of DM.

Phytochemicals	Concentration (%)	
	MSBE	ESBE
Alkaloids	16.33 ±0.88	-
Saponins	31.00 ±2.31	21.67 ±1.76
Steroids	-	-
Glycosides	-	-
Terpenoids	-	-
Flavonoids	21.01 ±2.33	38.01 ±1.16

Note: Values are in triplicate determinations ± SEM

The antibacterial activities of MSBE and ESBE showed varying degrees of inhibition (figure 1-3). The MSBE exhibited a maximum mean zone of inhibition against *S. aureus* (21.3 mm ±1.11 at 100 mg/mL) with its least inhibitory effect observed against *E. coli* (6.5 mm ±0.77) at 25 mg/mL. The extracts were ineffective against all the isolates at 6.25 mg/mL (figure 1-3). The ESBE demonstrated a maximum mean zone of inhibition against *S. typhi* (19.1 mm ±2.01 at 100 mg/mL concentrations) with no effect at 12.5 mg/mL and 6.25 mg/mL against *E. coli* and 6.25 mg/mL against both the *S. aureus* and *S. typhi*.

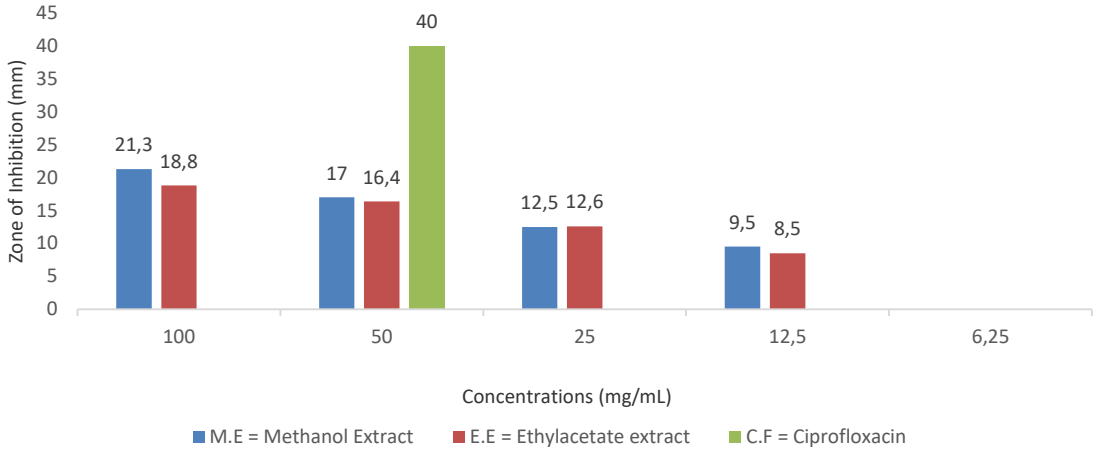

Figure 1. ZI of *S. aureus.*

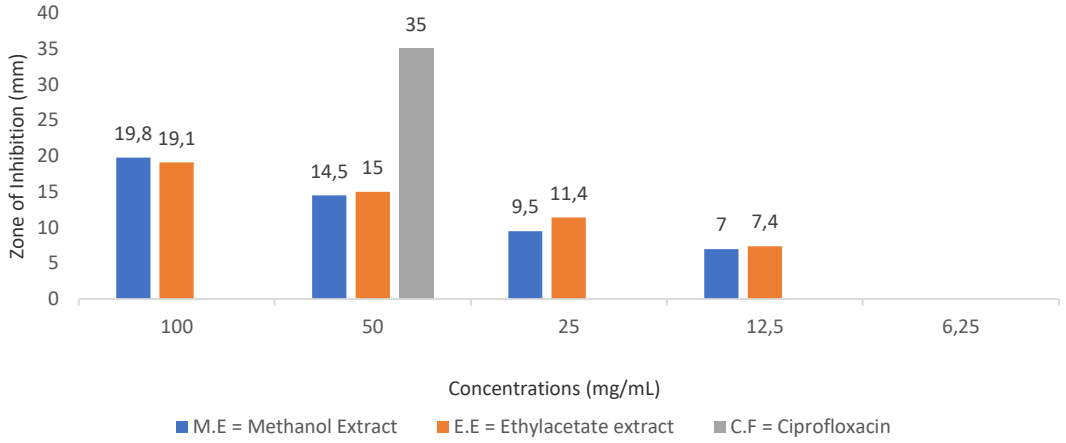

Figure 2. ZI of *S. typhi*.

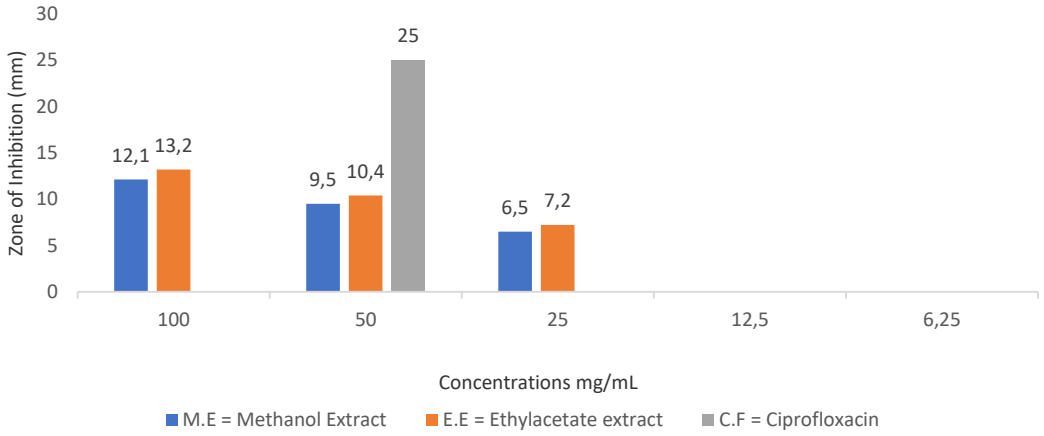

Figure 3. ZI of *E. coli*.

The inhibitory effects of MSBE and ESBE of DM are displayed in Table 3. MIC values for the MSBE and ESBE are between 100 mg/mL to 3.125mg/ml for *S. aureus*, *S. typhi*, and *E. coli*. *S. typhi* was the most sensitive to the MSBE and ESBE at 12.5 mg/mL and 25 mg/mL respectively.

Table 3. MIC of MSBE and ESBE.

Concentrations (mg/mL)	*S. aureus*		*S. typhi*		*E. coli*	
	M.E	**E.E**	**M.E**	**E.E**	**M.E**	**E.E**
100	-	-	-	-	-	-
50	-	-*	-	-	-	-*
25	-	+	-	-*	-*	+
12.5	-*	+	-*	+	+	+
6.25	+	+	+	+	+	+
3.125	+	+	+	+	+	+

Note: M.E= Methanol extract, E.E= Ethylacetate extract, * = MIC value, + = Turbidity , - = No turbidity

Table 4 displays the results of the MBC which reveal the minimum extract concentration needed to fully kill the bacterial isolates. A concentration of 12.5 mg/mL was required to kill *S. typhi* for the MSBE While 25 mg/mL was effective for the ESBE.

Table 4. MIC of MSBE and ESBE.

Test organism	MBC (mg/mL)	
	M.E	**E.E**
S. aureus	12.0 ±1.34	25.0 ±2.33
S. typhi	12.5 ±1.12	25.0 ±1.89
E. coli	25.0 ±2.21	50.0 ±2.62

Note: Values are in triplicate determinations ± SEM, M.E= Methanol extract, E.E= Ethylacetate extract

Discussion

The result indicated a variation in the presence of concentrations of phytochemicals in the MSBE and ESBE of DM which might be the difference in the polarity of the two solvents as the extraction process depends on the ability of the solvents to penetrate the extracts and solubilize the phytochemicals. Thus, different solvents extract different phytochemicals with variable concentrations (Aboshora *et al.*, 2014). This might also account for the detection of alkaloids in the MSBE as they are more polar. Flavonoids were previously reported in the ethyl acetate fraction of stem bark of DM with the absence of alkaloids and saponins which were all absent in the methanol extract including flavonoids (Ibrahim *et al.*, 2021). Our report agrees with a previous report (Abdullahi *et al.*, 2021; Mu'Azu *et al.*, 2022) for alkaloids, saponins, and flavonoids detection in MSBE of DM. The phytochemicals detected in our study were previously reported to exert antibacterial effects (Akinpelu *et al.*, 2014; Donadio *et al.*, 2021; Liu *et al.*, 2020; Yan *et al.*, 2021).

Alkaloids exert their antibacterial activities through the inhibition of bacterial cell walls, protein, nucleic acid, metabolic pathways, and by changing cellular membrane permeability (Yan *et al.*, 2021). Another mechanism of action for flavonoids (indole) was reported to be by inhibiting efflux pumps, biofilm formation, filamentous temperature-sensitive protein Z, and pyruvate kinase (Liu *et al.*, 2020). The positions of the nitrogen and methylenedioxy of isoquinoline were previously implicated in the antibacterial activity of the alkaloids (Qing *et al.*, 2017). Saponin fractions of Erythrophleum suaveolens exhibited antibacterial effects against some gram-positive cocci and gram-negative organisms indicating a good source of antibiotics (Akinpelu *et al.*, 2014). Flavonoids demonstrated antibacterial activity by inhibiting biofilm formation and attachment, nucleic acid synthesis, energy metabolism, membrane properties, and function, reducing pathogenicity (Xie *et al.*, 2015). Additionally, flavonoids further exhibit antibacterial effects by binding to microbial proteins required for basic cellular functions (Donadio *et al.*, 2021).

The antibacterial effects of the MSBE and ESBE were measured at 100, 50, 25, 12.5, and 6.25 mg/mL (figure 1-3). Both of the extracts demonstrated an antibacterial effect against the test organism with the highest mean ZI of 21.3 mm ±1.11 observed against *S. aureus* at 100 mg/mL by the MSBE. A similar result showed the superior effectiveness of the MSBE against *S. aureus* against the ESBE (Tiwari *et al.*, 2011). The ZI difference might be attributed to the solvent polarity which influences the types and concentrations of the phytochemical extracted, thus influencing the antibacterial effects of the extracts (Aboshora *et al.*, 2014; Gomashe *et al.*, 2014). The antibacterial effects demonstrated by DM might be attributed to growth-inhibiting phytochemicals such as flavonoids which were reported to possess antibacterial potentials (Sanusi *et al.*, 2022).

Although in the traditional and folkloric practice the primary solvent used for extraction is water for preparation, extraction with organic solvents exhibits superior antimicrobial effects compared to aqueous extracts (Gomashe *et al.*, 2014). The best practice for the extraction of broad-spectrum antimicrobial compounds employs alcohol as an extraction solvent as seen in the present study (Gomashe *et al.*, 2014). The effective concentration of the ESBE was against the *S. typhi* as presented in Table 1. The nature of the Gram-negative bacteria cell wall might contribute to the resistance of the organism to the extract by limiting the penetration of the extract to affect its action due to the thin lipopolysaccharide exterior membrane (Biswas *et al.*, 2013). However, the mesh-like peptidoglycan layer of Gram-positive bacteria allows for permeability and access to the extract (Malanovic & Lohner, 2016).

The MIC describes the minimum concentration of antimicrobial agent needed to inhibit microbial growth though not applicable in clinical practice for the determination of antibiotic dosage for a patient. However, it is applicable in the determination of the effective target antidiabetic with a lower chance of developing resistance (Wiegand *et al.*, 2008). The inhibitory effect of methanolic and ethyl acetate extracts DM was observed to be between 3.125mg/ml to 100 mg/ml for *S. aureus, S. typhi, and E. coli* with *S. typhi*

being the most sensitive. This variation in MBC values might be attributed to the phytoconstituents of the extract which vary due to solvent difference, thus, exhibiting inferior different inhibitory and bactericidal effects to the standard (Aboshora *et al.*, 2014; I. Abubakar & A. Usman, 2016; Gomashe *et al.*, 2014; Sanusi *et al.*, 2022). Similar results to the present study were reported on the phytoconstituents and antibacterial effect of stem bark extracts of DM (Sanusi *et al.*, 2022).

CONCLUSION

The study investigated the phytoconstituents and antibacterial effects of *D. microcarpum* which exhibited antibacterial effects against *E. coli*, *S. typhi*, and *S. aureus* with notable inhibitory and bactericidal effects. The antibacterial capabilities of *D. microcarpum* might be due to the phytochemical components of the plants and might be a potential source of a novel antibiotic against antibiotic-resistant bacteria.

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***Authors' Contributions*:** All authors contributed to the design and writing of the research. S.A.P. collected the plant sample and carried out the extraction while D.M.M. carried out the phytochemical analysis. A.A.M. carried out the antibacterial study.

***Competing Interests*:** The authors declare that there are no competing interests.

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REFERENCES

Abdullahi, A. R., Malami, S., & Bichi, L. A. (2021). In vivo antiplasmodial activity of detarium microcarpum (fabaceae) stem bark extract. *Thrita*, *10*(1), 1-9.

Aboshora, W., Lianfu, Z., Dahir, M., Qingran, M., Qingrui, S., Jing, L., Al-Haj, N. Q. M., & Ammar, A. (2014). Effect of extraction method and solvent power on polyphenol and flavonoid levels in Hyphaene thebaica L mart (Arecaceae)(Doum) fruit, and its antioxidant and antibacterial activities. *Tropical Journal of Pharmaceutical Research*, *13*(12), 2057-2063.

Abubakar, I., & Usman, A. (2016). Phytochemical and antibacterial investigations of moringa (Moringa oleifera) leaf extract on selected bacterial pathogens. *Journal of Microbiology and Antimicrobials*, *8*(5), 28-33.

Adhikari, B. (2021). Roles of alkaloids from medicinal plants in the management of diabetes mellitus. *Journal of Chemistry*, *2021*, 1-10.

Ahad, B., Shahri, W., Rasool, H., Reshi, Z. A., Rasool, S., & Hussain, T. (2021). Medicinal plants and herbal drugs: An overview. *Medicinal and Aromatic Plants: Healthcare and Industrial Applications*, 1-40.

Akinde, O., & Taiwo, M. (2017). Emerging antibiotic resistance in africa; threat to healthcare delivery. *MOJ Biology and Medicine*, *1*(4), 114-115.

Akinpelu, B. A., Igbeneghu, O. A., Awotunde, A. I., Iwalewa, E. O., & Oyedapo, O. O. (2014). Antioxidant and antibacterial activities of saponin fractions of Erythropheleum suaveolens (Guill. and Perri.) stem bark extract. *Scientific Research and Essays*, *9*(18), 826-833.

Andrews, J. M. (2005). BSAC standardized disc susceptibility testing method (version 4). *Journal of Antimicrobial Chemotherapy*, *56*(1), 60-76.

Biharee, A., Sharma, A., Kumar, A., & Jaitak, V. (2020). Antimicrobial flavonoids as a potential substitute for overcoming antimicrobial resistance. *Fitoterapia*, *146*, 104720.

Biradar, Y. S., Jagatap, S., Khandelwal, K. R., & Singhania, S. S. (2008). Exploring of antimicrobial activity of Triphala Mashi—An ayurvedic formulation. *Evidence-Based Complementary and Alternative Medicine*, *5*(1), 107-113.

Biswas, B., Rogers, K., McLaughlin, F., Daniels, D., & Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. *International journal of microbiology*, *2013*, 1-8.

Bouyahya, A., Dakka, N., Et-Touys, A., Abrini, J., & Bakri, Y. (2017). Medicinal plant products targeting quorum sensing for combating bacterial infections. *Asian Pacific Journal of Tropical Medicine*, *10*(8), 729-743.

Cheesbrough, M. (2002). Biochemical tests to identify bacteria. *District laboratory practice in tropical countries, Part*, *2*, 63-70.

De, N. B., & Ifeoma, E. (2002). Antimicrobial effects of components of the bark extract of neem (Azadirachta indica A. J uss). *Technology and Development*, *8*, 23-28.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. *Pharmaceutics*, *13*(5), 1-23.

Elton, L., Thomason, M. J., Tembo, J., Velavan, T. P., Pallerla, S. R., Arruda, L. B., Vairo, F., Montaldo, C., Ntoumi, F., & Abdel Hamid, M. M. (2020). Antimicrobial resistance preparedness in sub-Saharan African countries. *Antimicrobial Resistance & Infection Control*, *9*, 1-11.

Evans, W. C. (2009). *Trease and Evans' pharmacognosy*. Elsevier Health Sciences.

Fan, K., Ding, C.-F., Deng, S.-Y., Gao, W., Tan, B.-Y., Wu, H., Guo, Y., Song, J.-F., Zhang, L.-C., & Zhang, R.-P. (2022). Monoterpene indole N-oxide alkaloids from Tabernaemontana corymbosa and their antimicrobial activity. *Fitoterapia*, *158*, 105178.

Fang, Z., Li, J., Yang, R., Fang, L., & Zhang, Y. (2020). A Review: The Triterpenoid Saponins and Biological Activities of Lonicera Linn. *Molecules*, *25*(17), 3773.

George, B. P., Chandran, R., & Abrahamse, H. (2021). Role of phytochemicals in cancer chemoprevention: Insights. *Antioxidants*, *10*(9), 1455.

Gomashe, A. V., Gulhane, P. A., Junghare, M. P., & Dhakate, N. A. (2014). Antimicrobial activity of Indian medicinal plants: Moringa oleifera and Saraca indica. *International Journal of Current Microbiology and Applied Sciences*, *3*(6), 161-169.

Gupta, T., Kataria, R., & Sardana, S. (2022). A Comprehensive Review on Current Perspectives of Flavonoids as Antimicrobial Agent. *Current Topics in Medicinal Chemistry*, *22*(6), 425-434.

Harborne, A. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. springer science & business media.

Hossen, I., Hua, W., Ting, L., Mehmood, A., Jingyi, S., Duoxia, X., Yanping, C., Hongqing, W., Zhipeng, G., & Kaiqi, Z. (2022). Phytochemicals and inflammatory bowel disease: a review. *Critical Reviews in Food Science and Nutrition*, *60*(8), 1321-1345.

Ibrahim, J., Abdullahi, J., & Sule, M. O. (2021). Phytochemical and Antimicrobial Screening of the Stem-Bark and Leaf Extracts of Detarium Microcarpum (Leguminosae). *Central Asian Journal of Theoretical and Applied Science*, *2*(11), 130-138.

Jafaar, H. J., Isbilen, O., Volkan, E., & Sariyar, G. (2021). Alkaloid profiling and antimicrobial activities of Papaver glaucum and P. decaisnei. *BMC Research Notes*, *14*, 1-7.

Ji, Y., Thomas, C., Tulin, N., Lodhi, N., Boamah, E., Kolenko, V., & Tulin, A. V. (2016). Charon mediates immune deficiency–driven PARP-1–dependent immune responses in Drosophila. *The Journal of Immunology*, *197*(6), 2382-2389.

Jucá, M. M., Cysne Filho, F. M. S., de Almeida, J. C., Mesquita, D. d. S., Barriga, J. R. d. M., Dias, K. C. F., Barbosa, T. M., Vasconcelos, L. C., Leal, L. K. A. M., & Ribeiro, J. E. (2020). Flavonoids: biological activities and therapeutic potential. *Natural product research*, *34*(5), 692-705.

Lar, P. M., Ojile, E. E., Dashe, E., & Oluoma, J. N. (2011). Antibacterial Activity on Moringa Oleifera Seed Extracts on Some Gram Negative Bacterial Isolates. *African Journal of Natural Sciences*, *14*, 57-62.

Liu, Y., Cui, Y., Lu, L., Gong, Y., Han, W., & Piao, G. (2020). Natural indole-containing alkaloids and their antibacterial activities [https://doi.org/10.1002/ardp.202000120]. *Archiv der Pharmazie*, *353*(10), 2000120. https://doi.org/https://doi.org/10.1002/ardp.202000120

Maillard, J.-Y., Bloomfield, S. F., Courvalin, P., Essack, S. Y., Gandra, S., Gerba, C. P., Rubino, J. R., & Scott, E. A. (2020). Reducing antibiotic prescribing and addressing the global problem of antibiotic resistance by targeted hygiene in the home and everyday life settings: A position paper. *American Journal of Infection Control*, *48*(9), 1090-1099.

Malanovic, N., & Lohner, K. (2016). Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1858*(5), 936-946. https://doi.org/https://doi.org/10.1016/j.bbamem.2015.11.004

Mu'Azu, A. B., Baba, Y. B., Matinja, A. I., & Bukar, I. A. (2022). Haematological and lipid studies of methanol stem bark extract of detarium microcarpum in rats. *International Journal of Biomedical and Health Sciences*, *17*(2), 177-183.

Obadoni, B., & Ochuko, P. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of pure and applied sciences*, *8*(2), 203-208.

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. *Phytomedicine Plus*, *1*(2), 100029.

Qing, Z.-X., Yang, P., Tang, Q., Cheng, P., Liu, X.-B., Zheng, Y.-j., Liu, Y.-S., & Zeng, J.-G. (2017). Isoquinoline Alkaloids and Their Antiviral, Antibacterial, and Antifungal Activities and Structure-activity Relationship. *Current Organic Chemistry*, *21*(18), 1920-1934. https://doi.org/10.2174/1385272821666170207114214

Rahayu, Y. Y. S., Araki, T., & Rosleine, D. (2020). Factors affecting the use of herbal medicines in the universal health coverage system in Indonesia. *Journal of Ethnopharmacology*, *260*, 112974.

Sanusi, S. B., Lawal, S. M., Usman, A., Musa, F. M., Ardo, B., & Way, T. B. (2022). Phytochemical Analysis and Antibacterial Activity of Stem Bark Extracts of Detarium Microcarpum Against Bacteria Causing Gastrointestinal Tract Infections in Humans. *Dutse Journal of Pure and Applied Sciences*, *8*(1b). https://doi.org/10.4314/dujopas.v8i1b.10

Song, F., Liu, D., Huo, X., & Qiu, D. (2022). The anticancer activity of carbazole alkaloids. *Archiv der Pharmazie*, *355*(1), 2100277.

Talaiekhozani, A. (2013). Guidelines for quick application of biochemical tests to identify unknown bacteria. *Account of Biotechnology Research (2013)*.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.

Tm, C., Pi, S. J., Negri, G., Rm, N., & Rz, M. (2022). Antimicrobial activity of flavonoids glycosides and pyrrolizidine alkaloids from propolis of Scaptotrigona aff. postica. *Toxin Reviews*, 1-16.

Tong, C., Zou, W., Ning, W., Fan, J., Li, L., Liu, B., & Liu, X. (2019). Correction: Synthesis of DNA-guided silver nanoparticles on a graphene oxide surface: enhancing the antibacterial effect and the wound healing activity. *RSC advances*, *9*(67), 39434-39434.

Ullah, R., Alqahtani, A. S., Noman, O. M. A., Alqahtani, A. M., Ibenmoussa, S., & Bourhia, M. (2020). A review on ethno-medicinal plants used in traditional medicine in the Kingdom of Saudi Arabia. *Saudi Journal of Biological Sciences*, *27*(10), 2706-2718. https://doi.org/https://doi.org/10.1016/j.sjbs.2020.06.020

Wiegand, I., Hilpert, K., & Hancock, R. E. W. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature Protocols*, *3*(2), 163-175.

Wu, M., Ni, L., Lu, H., Xu, H., Zou, S., & Zou, X. (2020). Terpenoids and their biological activities from Cinnamomum: a review. *Journal of Chemistry*, *2020*, 1-14.

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. *Current Medicinal Chemistry*, *22*(1), 132-149. https://www.ingentaconnect.com/content/ben/cmc/2015/00000022/00000001/art00012

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review. *Antibiotics*, *10*(3), 1-30.

Yang, M., Hao, Z., Wang, X., Zhou, S., Zhu, D., Yang, Y., Wei, J., Li, M., Zheng, X., & Feng, W. (2022). Neocornuside A–D, Four Novel Iridoid Glycosides from Fruits of Cornus officinalis and Their Antidiabetic Activity. *Molecules*, *27*(15), 4732.

Yang, W., Chen, X., Li, Y., Guo, S., Wang, Z., & Yu, X. (2020). Advances in pharmacological activities of terpenoids. *Natural Product Communications*, *15*(3), 1934578X20903555.

Zheng, Z., Zhang, L., & Hou, X. (2022). Potential roles and molecular mechanisms of phytochemicals against cancer. *Food & Function*, *13*(18), 9208-9225.