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RESEARCH ARTICLE

Variation of Phytochemicals and Antimicrobial Activity of *Terminalia brownii*

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ABSTRACT

This study screened the variation of phytochemicals in *Terminalia brownii* leaves, bark and root extracts and evaluated their microbial activity against *Escherichia coli* and *Staphylococcus aureus*. Preliminary phytochemical screening showed the presence of tannins, alkaloids, saponins and steroids in leaves, bark and roots; terpenoids in barks and roots, while flavonoids were absent. Leaf, bark and root extracts significantly ($p < 0.05$) inhibited *E. coli* and *S. aureus* growth. Root extracts (methanol and ethanol) had significantly higher zones of inhibition (18.0 and 14.6 mm) against *E. coli* compared to the other plant parts. Similarly, leaf and root extracts produced higher zones of inhibition of 12.3 and 17.4 mm against *S. aureus,* respectively. Data from this study shows that *T. brownii* is a good candidate for further pharmaceutical investigations and could be exploited for the development of natural bioactive agents to improve human health.

Keywords: Screening, Extracts, Inhibition, Bioactive, Evaluation.

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INTRODUCTION

Antimicrobial resistance (AMR) has become a major challenge to the health sector in Sub-Saharan African countries, including Kenya. Lower and middle-income countries are already threatened due to high level of AMR compared to higher-income countries.[1] The situation of AMR in these countries is on an upward trend due to the indiscriminative use of antibiotics, over-the-counter acquisition without prescriptions, and readily available substandard drugs.[2,3] Inadequate provision of clean water, high population density, lack of proper sanitation and poor infrastructure in developing countries promotes rapid spread of infectious diseases leading to high demand of antibiotics. Similarly, poor governance and reduced expenditure in public health sector increase the prevalence of AMR bacteria.[4] Resistance to antibiotics by bacteria caused hundreds of thousands of death in Sub-Sahara Africa especially in countries with weak health systems. According to Urban-Chmiel *et al.,*[5] antibiotic resistance is growing very fast on commonly used antibiotics and last resort drugs.

Resistance of bacteria to antibiotics can be natural, where the species or strains of certain bacteria lack susceptibility to drugs. This can be due to the impermeability of the cell wall, absence of appropriate receptors, low affinity to the antibiotic or production of antibiotic-inactivating enzymes.[5] Lack of susceptibility of bacteria to antibiotics can be classified as primary or secondary. Primary resistance occurs due to spontaneous mutation which takes place without exposure to antibiotics. Resistance is embedded in the chromosome of the bacteria and cannot be transmitted to other species of bacteria.[5,6] Once the bacteria acquire resistance genes due to mutation, they are able to survive and outnumber other susceptible bacteria in the population. Secondary resistance occurs when the bacteria species come in contact with the antibacterial agent or drug. This type of resistance is extra-chromosomal as the genes involved are located in a plasmid.[7] The plasmid may contain a few or many genes that confer resistance to several antibiotics. These resistant genes can be transferred from one species to another through conjugation or transduction.[5]

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Increased antimicrobial resistance has resulted in fewer options for treating patients, leading to high morbidity and mortality. Antibiotic resistance by strains of *Staphylococcus aureus* and *Escherichia coli* has become rampant[8] hence the need for new sources of antimicrobial agents. Plants harbor natural products that enable them to resist microbial pathogens which can be used as antimicrobial sources but have been neglected in favor of synthetic antimicrobials. Currently, plants are receiving more attention as a source of antimicrobial compounds as they are readily available, safe to humans and environmentally friendly as their products are easily broken down.[9,10] They synthesize a variety of biologically active metabolites such as terpenoids, tannins, iridoids, alkaloids, coumarins, saponins, lignans, flavonoids, steroidal and xanthones which have antimicrobial properties.[9,11] Extracts from some medicinal plants such as orchids,[12] *Tamarindus indica*[13] and roselle (*Hibiscus sabdariffa*)[14] have therapeutic and antimicrobial activity against bacterial pathogens such as *E. coli* and *Shigella* species. Similarly, *Psidium guajava* L. have shown an inhibitory effect against clinical isolates *Proteus* spp*., Staphylococcus* spp. and *Pseudomonas aeruginosa*.[3] *Terminalia brownii* plant belonging to the combretaceae family is used traditionally in the management of different human illness such as yellow fever, ulcers, hepatitis and diabetes. The species colonizes a wide range of ecological zones where it has been used in traditional medicine.[15] *T. brownii* extracts have shown antimicrobial activity against a variety of bacteria and fungi in different ecological zones[15,16] but the antimicrobial activity of the species growing in western Kenya against *E. coli* and *S. aureus* is still unknown. This study was carried out to determine the phytochemical and susceptibility of *E. coli* and *S. aureus* on *T. brownii* leaf, bark and root extracts.

MATERIALS AND METHODS

Sample Collection and Processing

Fresh healthy leaves, bark and roots of *T. brownii* plant were collected around Jaramogi Oginga University of Science and Technology in ziplock bags and taken to the microbiology laboratory. They were thoroughly washed with running water to remove debris and dust particles, rinsed using distilled water, and left to dry at room temperature in the shade. Dried plant parts were separately ground into fine powder using mortar and pestle and stored in a cool and dry place. Total extraction of each plant part was done in ethanol and methanol solvent in the ratio of 1: 10 (plant material/solvents). Twenty grams of each plant part powder was separately soaked in 200 mL solvent in conical flasks for at least 24 hours. The extracts were filtered using Whatman No. 1 filter paper and concentrated under a vacuum in a rotary evaporator at 40°C. The crude extracts were further dried by evaporation by placing a beaker containing the extract on open place for several days until the extracts were completely dry. The plant extracts that were not used immediately were stored at 4°C.[17]

Sterility Test of the Plant Extracts

Each methanol and ethanol extract was tested for its sterility from microbes by inoculating 0.5 mL of extract on Mueller Hinton Agar and incubated at 37°C for 24 hours. Absence of any growth from the extract after incubation indicated sterility.[18]

Phytochemical screening

Phytochemicals of interest in the plant's leaves, roots and bark were screened using standard protocols.

Tannins

The presence of tannins was determined by boiling 0.5 gm of the extract with 5 mL of ethanol for 5 minutes. The filtrate (1-mL) was diluted with distilled water and two drops of ferric chloride were added dropwise. A color change from greenish to black indicated the presence of tannins.[19]

Flavonoids

This was done by dissolving 0.5 mg of the extracts in 0.2 NaOH and a color change from yellow to colorless after the addition of drops of H_2SO_4 indicated that flavonoids were present.[20]

Alkaloids

Alkaloids were determined by dissolving 0.5 gm of extracts in warmed 2% H_2SO_4 for two minutes before filtering. Few drops of Mayer reagent were added. The appearance of a creamy white precipitate indicated the presence of alkaloids.[19]

Saponins

Using the method previously described by Gul *et al.,*[20] 0.5 gm of each extract was dissolved in 1-mL of methanol then filtered. Distilled water was added, shaken for a few minutes and a persistent frothing indicated presence of saponins.

Steroids

The presence of steroids was determined by dissolving 0.5 gm of each extract in chloroform plus anhydrous acetic acid and heating then cooled. Concentrated H_2SO_4 was added through the walls of the tube dropwise. The formation of brown ring indicated the presence of steroids.[21]

Terpenoids

A 0.5 gm extract was mixed with a few drops of acetic anhydride then boiled and cooled. Drops of concentration H_2SO_4 were added from the sides of the tube. Formation of red colour in the lower region indicated that terpenoids are present.[18]

Antimicrobial Assay

Culture media and test microorganisms

Culture media Muller Hinton Agar (MHA) and Muller Hinton Broth as well test bacteria, *E. coli* (ATCC25922) and *S. aureus* (ATCC 25923) were obtained from the Microbiology laboratory of Jaramogi Oginga University of Science and Technology. Culture media was prepared according to the manufacturer's instructions.

Antimicrobial Activity Evaluation by disc diffusion

Colonies (3-4) of previously cultured bacterial pathogens were

inoculated separately in 4 mL peptone broth. The individual bacteria suspension density was adjusted to 0.5 McFarland turbidity standard, resulting in a 1.5 × 108 CFU concentration. Discs were prepared by punching 2mm discs from Whatman's No 3 filter paper and then sterilized. Extracts from plant parts were reconstituted in DMSO and used to make 100 mg/mL concentrations, 50 and 25 mg/mL. The discs were soaked in the extracts before being used.[18]

Mueller Hinton agar plates were seeded with bacteria suspension using sterile cotton swabs and left in lamina flow for 5 minutes. Discs were transferred from the extracts using sterile forceps and placed on the surface of the plates with bacteria and plates sealed with laboratory film. Plated with discs containing DMSO only were used as negative control. They were incubated at 37ºC for 24 hours in a completely randomized design and zone of inhibition was measured.[3]

Data Analysis

Triplicate data was subjected to analysis of variance (ANOVA) in SAS software (SAS Institute, version 9.1) and where significant, means were separated using Fisher's Least Significant Difference at $p \leq 0.05$.

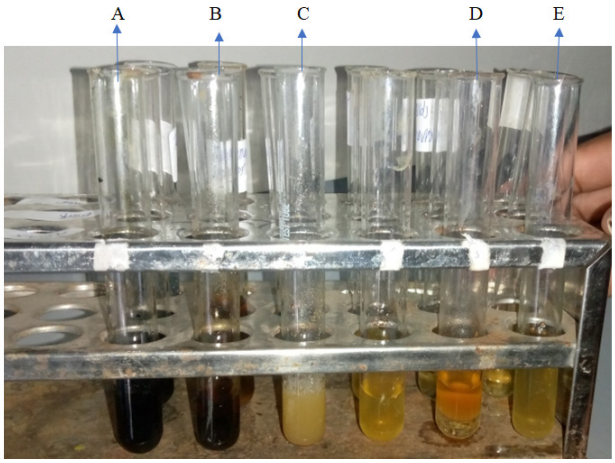
RESULTS

Phytochemical Screening:

Tannins and alkaloids were present in all the plant parts in high and moderate concentrations respectively (Table 1 and Figure 1). The bark had a higher concentration of saponins (leaves), steroids (roots) and terpenoids. A moderate concentration of saponins was present in the roots and bark, steroids (leaves and bark) and terpernoids in the roots. Flavonoids were absent in all three plant parts compared to terpernoids that was only present in the barks and roots.

Evaluation of Antimicrobial Activity

The mean growth inhibition of ethanol extracts against *E. coli and S. aureus* ranged from 11.6 to 18.0 mm and 12.3 to 17.4 mm compared methanol extracts ranged between 10.6 to 14.6 mm and 10.7 to 12.3 mm, respectively (Table 2). Root



Key: (A) Tannins present; (B) Flavonoids absent; (C) Alkaloids present; (D) Steroids present; (E) Terpenoids absent

Figure 1: Phytochemical screening of bark

ethanol extracts had significantly higher zone of inhibition ($p < 0.05$) against *E. coli* and *S. aureus* compared to methanol extracts. Mean zone of inhibition of leaves and stem ethanol and methanol extracts were not significantly ($p > 0.05$) different against *E. coli*. Leaves ethanol and methanol extracts significantly ($p < 0.05$) inhibited the growth of *S. aureus* while there were no significant differences between stem ethanol and methanol extracts.

The mean zones of inhibition of different concentrations of both methanol and ethanol extracts against *E. coli* and *S. aureus* were significantly ($p < 0.05$) different (Table 3). Mean zone of inhibition of methanol extracts against *E. coli* and *S. aureus* ranged from 8.3 to 12.3 mm and 7.3 to 10.0 mm, while for ethanol extracts, it ranged between 9.0 to 14.7 mm, respectively at 25 mg/mL. The highest concentration (75 mg/mL) of extracts from the three plant parts produced higher mean zones of inhibition against *E. coli* and *S. aureus* compared to other concentrations in both solvents. At 75 mg/mL methanol extracts, the mean zone of inhibition of *E.coli and S. aureus* ranged between 14.0 to 17.0 mm and 13.0 to

Table 1: Phytochemical profiles in *T. brownii* plant extracts

Plant parts	Tannins	Flavonoids	Alkaloids	Saponins	Steroids	Terpenoids
Leaves	++	-	+	++	+	-
Bark	++	-	+	+	+	++
Root	++	-	+	+	++	+

Key: indicates absence of phytochemicals; + indicates presence with moderate concentration; ++ indicates presence with high concentration**.**

Table 2: Overall effect of plant parts and solvent extracts on growth inhibition of *E. coli* and *S. aureus*

	E. coli			*S. aureus*		
Plant parts	Ethanol	Methanol	*p-value*	Ethanol	Methanol	*p-value*
Leaves	11.6a	10.6a	0.12	12.3a	10.7b	0.02
Bark	12.3a	13.3a	0.14	9.6a	10.3a	0.08
Root	18.0a	14.6b	0.01	17.4a	12.3b	<.0001

Means followed by the same letters across the rows are not significantly different at P = 0.05

Table 3: Mean zone of inhibition (mm) of *T. brownii* extracts on growth of *E. coli* and *S. aureus*

Treatment and solvent											
		Methanol extracts					*Ethanol extracts*				
Plant parts	*Organism*	*DMSO*	*25 mg/mL*	*50 mg/mL*	*75 mg/mL*	*p-value*	*DMSO*	*25 mg/mL*	*50 mg/mL*	*75 mg/mL*	*p-value*
Leaves	*E. coli*	0.0^{c}	8.3^{b}	9.7^{b}	14.0^{a}	0.003	0.0^{d}	9.3^{c}	11.7^{b}	14.0^{a}	0.0062
	S. aureus	0.0^{c}	8.3^{b}	11.0^{a}	13.0^{a}	0.015	0.0^{d}	7.3^{c}	9.6^{b}	12.0^{a}	0.0005
Bark	*E. coli*	0.0^{d}	10.3^{c}	13.3^{b}	16.3^{a}	0.002	0.0^{d}	9.0^{c}	12.3^{b}	15.7^{a}	0.0046
	S. aureus	0.0^{d}	7.3^{c}	10.7^{b}	13.0^{a}	0.014	0.0^{d}	10.3^{c}	12.3^{b}	14.3^{a}	0.0008
Root	*E. coli*	0.0^{d}	12.3^{c}	14.7^{b}	17.0^{a}	0.003	0.0^{c}	14.7^{b}	18.0^{ab}	21.3^{a}	0.0246
	S. aureus	0.0^{d}	10.0^{c}	12.0^{b}	15.0^{a}	0.003	0.0^{d}	15.3^{c}	17.3^{b}	19.7^{a}	0.0003

Means followed by the same letters across the rows are not significantly different at P = 0.05

15.0 mm, respectively. For ethanol extracts the mean zone of inhibition ranged from 14.0 to 21.3 mm and 12.0 to 19.7 mm for *E. coli and S. aureus* respectively. The highest zones of inhibition on *E. coli* (21.3 mm) and *S. aureus* (19.7 mm) were recorded in root ethanol extracts.

DISCUSSION

Leaves, bark and roots of T. *brownii* contained tannins, alkaloids, saponins and steroids while terpenoids were present in the bark and roots. Flavonoids were absent in all the three plant parts. These results are in agreement with a previous study by Ikikii *et al.*[15] that reported the presence of flavonoids in the flowers of flowers of *T. brownii*. However, Periasamy, Alemayehu[22] recoded the presence of flavonoids in methanolic leaf extracts. The difference could be attributed to differences ecological and geographical locations where plants were collected that affected the presence of phytochemicals. Environmental conditions such as drought and altitude influences the type and quantity of phytochemical present in the plant.[23,24] In addition, other factors such as acidity or salinity have also been reported to contribute to variation in the concentrations of phytochemicals across different regions in Kenya.[25] The presence of Phytoconstituents in plants have received a lot of interest in the recent past in the advancement of modern medicine by exploring new sources of therapeutic compounds.[26] Terpenoids are useful in treatment and prevention of diseases as they have antibacterial, antifungal, antiparasitic, antiviral and anti-inflamatory activities. Steroids are well known for soothing inflamed airway in asthma as well as reducing cholesterol.[27] Plants are reservoirs of phytochemicals with therapeutic properties that are active against a wide range of microbes at low concentration. Similarly, phytochemicals display antioxidant properties which is of great value in lowering oxidative stress which is a major contributor to development of high risk diseases in human.[27,28] Extracts from *T. brownii* demonstrated antibacterial activity against *S. aureus* and *E. coli*. The wider zones of inhibition observed in root extracts (methanol and ethanol) against the two bacteria could be due to the high concentration of steroids and tannins compared to other parts. The results are consistent with those reported by Ikikii *et al.*,[15] in which methanolic extracts from *T. brownii* exhibited inhibitory effects against *E. coli, P. aeroginosa* and *S. aureus.* Tannins are comprised of polyphenolic compounds which inhibit microbial proliferation by binding and precipitating proteins,[29] as well as directly attacking cellular organelles and cell membranes.[30] Steroids function as antiviral, antifungal, antibacterial and antioxidant with high efficacy against *S. aureus* and *Acinetobacter baumannii*.[31] Ethanol extracts exhibited greater efficacy compared to methanol extract and this could be attributed to the fact that ethanol is less polar compared to methanol which extracted more of non polar compounds with antimicrobial activity. The results are in agreement with the report of[15,32] that ethanol extracts are active against S. *aureus*. Antimicrobial properties of plant extracts are due to their ability to bind to membrane proteins through hydrophobic and hydrogen bonds. The binding of proteins alters membrane permeability thus affecting the exchange of important material between the environment and the cell.[32] Methanol and ethanol extracts inhibited *E. coli* more compared to *S. aureus* in both methanol and ethanol extracts. This could be attributed to the presence of high content of peptidoglycan layer *S. aureus* which hindered the penetration of the extracts into the cell to arrest metabolic processes. Plant extracts can inhibit normal cell communication; quorum sensing (QS) vital in responding to environmental changes. Some plant compounds modifies or completely inhibit protein-protein interactions thus lowering or altering vital cell metabolic functions.[33] They also inhibit bacterial growth through interference with intermediary metabolism, coagulation of cytoplasmic constituents and inhibition of biofilm formation that protects the pathogen during infection.[33,34]

CONCLUSION

The three parts of *T. brownii* contained tannins, alkaloids, saponins, steroids and terpenoids while flavonoids were absent in all the three plant parts. The leaves, bark and roots extracts inhibited the growth of *E. coli* and *S. aureus* with root extracts being more active than other parts. Thus medicinal plants including *T. brownii* are potential sources of new antimicrobial

agents.

CONFLICT OF INTEREST

We declare that there is no any conflict of interest among authors.

AUTHOR'S CONTRIBUTION

All authors contributed equally in the design, laboratory experiments, data analysis and manuscript writing.

REFERENCES

1. Gitaka J, Kamita M, Mureithi D, Ndegwa D, Masika M, Omuse G, *et al.* Combating antibiotic resistance using guidelines and enhanced stewardship in Kenya: a protocol for an implementation science approach. BMJ open 2020;10(3):e030823. doi:10.1136/bmjopen-2019-030823
2. Maina M, Mwaniki P, Odira E, Kiko N, McKnight J, Schultsz C, *et al.* Antibiotic use in Kenyan public hospitals: Prevalence, appropriateness and link to guideline availability. International Journal of Infectious Diseases 2020;99:10-18. https://doi.org/10.1016/j.ijid.2020.07.084
3. Owusu E, Ahorlu MM, Afutu E, Akumwena A, Asare GA. Antimicrobial activity of selected medicinal plants from a sub-Saharan African country against bacterial pathogens from post-operative wound infections. Medical Sciences 2021;9(2):23. https://doi.org/10.3390/medsci9020023
4. Omulo S, Lofgren ET, Lockwood S, Thumbi SM, Bigogo G, Ouma A, *et al.* Carriage of antimicrobial-resistant bacteria in a high-density informal settlement in Kenya is associated with environmental risk-factors. Antimicrobial Resistance & Infection Control 2021;10(1):1-12. https://doi.org/10.1186/s13756-021-00886-y
5. Urban-Chmiel R, Marek A, Stępień-Pyśniak D, Wieczorek K, Dec M, Nowaczek A, *et al.* Antibiotic resistance in bacteria—A review. Antibiotics 2022;11(8):1079. https://doi.org/10.3390/antibiotics11081079
6. Hasan TH, Al-Harmoosh RA. Mechanisms of antibiotics resistance in bacteria. Sys Rev Pharm 2020;11(6):817-823.
7. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Virulence mechanisms of bacterial pathogens 2016:481-511. doi:10.1128/microbiolspec.VMBF-0016-2015
8. Reygaert WC. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS microbiology 2018;4(3):482. DOI: 10.3934/microbiol.2018.3.482
9. Atwaa ESH, Shahein MR, Radwan HA, Mohammed NS, Aloraini MA, Albezrah NKA, *et al.* Antimicrobial Activity of Some Plant Extracts and Their Applications in Homemade Tomato Paste and Pasteurized Cow Milk as Natural Preservatives. Fermentation 2022;8(9):428. https://doi.org/10.3390/fermentation8090428
10. Faye G, Birhanu T, Belete T. Survey and antimicrobial activity study of ethnomedicinal plants in selected districts of North Shewa Zone, Oromia, Ethiopia. Infection and Drug Resistance 2021:5511-5520. doi: 10.2147/IDR.S333772
11. Egamberdieva D, Wirth S, Behrendt U, Ahmad P, Berg G. Antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. Frontiers in microbiology 2017;8:199. doi: 10.3389/fmicb.2017.00199
12. Chagona P, Kwamboka N, Gaya H, Makonde H, Adem A, Osano K, *et al.* Phytochemical Analysis and Antibacterial Activity of the Kenyan Wild Orchids. Micro Environer 2021;1(02):93-100. https://doi.org/10.54458/mev.v1i02.6677
13. Lim H-W, Seo K-H, Chon J-W, Song K-Y. Antimicrobial activity of *Hibiscus sabdariffa* L.(Roselle) powder against food-borne pathogens present in dairy products: Preliminary study. Journal of Dairy Science and Biotechnology 2020;38(1):37-44. https://doi.org/10.22424/jdsb.2020.38.1.37
14. Prasetyoputri A, Rahmawati S, Atikana A, Izzati F, Hapsari Y, Septiana E, *et al.* A Mini Review on the Antibacterial Activity of Roselle (*Hibiscus sabdariff*a L.) Phytochemicals. In: IOP Conference Series: Materials Science and Engineering; 2021: IOP Publishing; 2021. p. 012017. **DOI 10.1088/1757-899X/1192/1/012017**
15. Ikikii SM, Odalo JO, Omondi AO, Kahindo JM. Phytochemical screening and antimicrobial activity of flowers of *Terminalia brownii*. Journal of Pharmacognosy and Phytochemistry 2022;11(5):24-27. **DOI:** https://doi.org/10.22271/phyto.2022.v11.i5a.14499
16. Salih EYA, Kanninen M, Sipi M, Luukkanen O, Hiltunen R, Vuorela H, *et al.* Tannins, flavonoids and stilbenes in extracts of African savanna woodland trees *Terminalia brownii, Terminalia laxiflora* and *Anogeissus leiocarpus* showing promising antibacterial potential. South African Journal of Botany 2017;108:370-386. http://dx.doi.org/10.1016/j.sajb.2016.08.020
17. Keshebo DL, Washe AP, Alemu F. Determination of antimicrobial and antioxidant activities of extracts from selected medicinal plants. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS) 2016;16(1):212-222.
18. Kebede T, Gadisa E, Tufa A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS One 2021;16(3):e0249253. https://doi.org/10.1371/journal.pone.0249253
19. Sheel R, Nisha K, Kumar J. Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR Journal of Applied Chemistry 2014;7(1):10-13.
20. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. The Scientific World Journal 2017;2017. https://doi.org/10.1155/2017/5873648
21. Setyawati I, Wijayanti N, Wiratmini N. Phytochemical content, extract standardization and antioxidant activity of *Calliandra calothyrsus* Meissn leaf, a potential phytoestrogen source. In: IOP Conference Series: Earth and Environmental Science; 2019: IOP Publishing; 2019. p. 012075. http://dx.doi.org/ 10.1088/1755-1315/347/1/012075
22. Periasamy G, Alemayehu Y, Tarekegn W. Evaluation of in vivo central analgesic activity and prelimnary phytochemical screening of methanolic extract of *T. brownii* leaves. Int J Pharm Biol Sci 2015;5(4):49-53.
23. Samaniego I, Espin S, Cuesta X, Arias V, Rubio A, Llerena W, *et al.* Analysis of environmental conditions effect in the phytochemical composition of potato (*Solanum tuberosum*) cultivars. Plants 2020;9(7):815. doi:10.3390/plants9070815
24. Biondi F, Balducci F, Capocasa F, Visciglio M, Mei E, Vagnoni M, *et al.* Environmental conditions and agronomical factors influencing the levels of phytochemicals in Brassica vegetables responsible for nutritional and sensorial properties. Applied Sciences 2021;11(4):1927. doi.org/10.3390/app11041927
25. Gaya C, Kawaka J, Muchugi A, Ngeranwa J. Variation of alkaloids

in the Kenyan *Zanthoxylum gilletii* (De wild Waterman). African Journal of Plant Science 2013;7(9):438-444.
26. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, *et al.* Antibiotic resistance: a rundown of a global crisis. Infection and drug resistance 2018;11:1645. doi: 10.2147/IDR.S173867
27. Maharaj A, Naidoo Y, Dewir YH, Rihan H. Phytochemical Screening, and Antibacterial and Antioxidant Activities of Mangifera indica L. Leaves. Horticulturae 2022;8(10):909. https://doi.org/10.3390/horticulturae8100909
28. Bouyahya A, Et-Touys A, Bakri Y, Talbaui A, Fellah H, Abrini J, *et al.* Chemical composition of Mentha pulegium and *Rosmarinus officinali*s essential oils and their antileishmanial, antibacterial and antioxidant activities. Microbial pathogenesis 2017;111:41-49. DOI: 10.1016/j.micpath.2017.08.015
29. Kaczmarek B. Tannic acid with antiviral and antibacterial activity as a promising component of biomaterials—A minireview. Materials 2020;13(14):3224. doi: 10.3390/ma13143224
30. Pereira AV, Góis MB, Azevêdo TKB, Ferraz FN, Vieira SLV, Cavalcanti-Dantas VM, *et al.* Effects of associations of tannins from *Anacardium occidentale* and *Anadenanthera colubrina* with cephalosporin against bovine *Staphylococcus aureus* isolates. Arquivos do Instituto Biológico 2018;85. DOI:10.1590/1808-1657000582016
31. Vollaro A, Esposito A, Antonaki E, Iula VD, D'Alonzo D, Guaragna A, *et al.* Steroid derivatives as potential antimicrobial agents against *Staphylococcus aureus* planktonic cells. Microorganisms 2020;8(4):468. DOI:10.3390/microorganisms8040468
32. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, *et al.* Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in microbiology 2018;9:1639. doi: 10.3389/fmicb.2018.01639
33. Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms 2021;9(10):2041. https://doi.org/10.3390/microorganisms9102041
34. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrobial Resistance & Infection Control 2019;8(1):1-28. https://doi.org/10.1186/s13756-019-0559-6