BACTERIAL EMPIRE

2022, VOL. 5, NO. 4, e461

OPEN ACCESS

SCICELL

ANTIBACTERIAL ACTIVITIES OF ETHANOL EXTRACTS FROM *Croton macrostachyus* (Hochst. Ex Delile) AND *Justicia schimperiana* (Hochst. Ex Nees) BARK AND LEAF AGAINST CERTAIN PATHOGENIC BACTERIA

Lire Dindamo, Sasikumar J. M., Andualem Getachew and Ameha Kebede*

Address (es): Dr. Jagathala Mahalingam Sasikumar,
School of Biological Sciences and Biotechnology, Haramaya University, Ethiopia.

*Corresponding author: jmsashikumar@gmail.com

https://doi.org/10.36547/be.461

ABSTRACT

Our study was designed to evaluate antibacterial activities of ethanol extracts from *Croton macrostachyus* and *Justicia schimperiana* bark and leaf against certain bacterial pathogens such as *E. coli* O157 H:7 ATCC 25922, *Salmonella* Typhi ATCC 13311, *Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 196151. Qualitative and quantitative methods were followed to screen the phytochemicals in the plants extracts and to estimate total phenolic content (TPC) and terpenoids. Antibacterial activity was evaluated by disc diffusion method and by determination of Minimum Inhibitory Concentration (MIC). The results of the investigation revealed that both the plants were found to contain alkaloids, saponin, steroids, tannins and terpenoids. Highest TPC (3.82±0.82 mg/g Tannic Acid Equivalent) and highest Terpenoid content (118.33±4.4 mg/g) were observed in the leaf extract of *C. macrostachyus*. Bark extract of *C. macrostachyus* displayed the strongest activity against the gram-positive bacteria *S. aureus* with an inhibition zone of 16.17±0.67 mm. *E. coli* O157 H:7 and *S.* Typhi were found more resistant to bark extract of *J. schimperiana* at the concentration of 100 mg/ml. The potent plant part extract should be subjected for further phytochemical analysis and bioactive investigations.

Keywords: Pathogenic bacteria, gram positive, gram negative, minimum inhibitory concentration, phytochemicals, *Croton macrostachyus*, *Justicia schimperiana*.

INTRODUCTION

The infections caused by bacterial pathogens that are resistant to drugs are a foremost cause of mortality across the world. The antimicrobial resistance of bacteria led to death of 1.27 million people around the world in 2019 (**Antimicrobial Resistance Collaborators, 2022**). Among the bacterial pathogens, *Salmonella* Typhi (**Ali Shah *et al*., 2020**), *Staphylococcus aureus* (**Foster, 2017; Guo *et al*., 2020**), *Streptococcus pyogenes* (**Pieretti *et al*., 2017**) have emerged highly resistant to the antibacterial drugs (**Ali Shah *et al*., 2020**). Further, several side effects of the antibacterial drugs have been identified (**Tsuruga, 2007**). Hence, researchers have been hunting for alternative medicine from plants as antibacterial agents (**Sasikumar *et al*., 2005, Hassan *et al*., 2014, Zewditu *et al*., 2022, Isunu *et al*., 2022**). Thus, our research focussed on the investigation of ethanol extracts from *Croton macrostachyus* and *Justicia schimperiana* bark and leaf against certain bacterial pathogens.

The plant *C. macrostachyus* is used in Ethiopian traditional medicine for the treatment of various ailments (**Giday 2007, Teklehaimanot and Giday, 2007, Mechesso *et al*., 2016**). Cytotoxic diterpenes were isolated from the seeds of this plant (**Yong *et al*., 2017**). Some phenolic compounds were also obtained (**Tala *et al*., 2013**). A few studies were reported on antibacterial activity of the plant (**Geyid *et al*., 2005; Mesfin *et al*., 2010; Tene *et al*., 2009; Wagate *et al*., 2010**). The leaves and stem bark of *J. schimperiana* are used to treat wound, gonorrhoea, malaria, rabies, a burning and arthritis, jaundice, and headache (**Habtamu *et al*., 2014, Tamrat *et al*., 2015**). Antidiarrheal activity of the leaf extract of this plant was investigated (**Mekonnen *et al*., 2018**). A study from Ethiopia reported the hepatoprotective activity of *J. schimperiana* (**Umer et al., 2010**). Earlier, essential oil from leaves of *J. schimperiana* were tested for antibacterial activity (**Abebe *et al*., 2018**). Despite some antibacterial activities of *C. macrostachyus* and *J. schimperiana* from different areas in the country and overseas, environmental factors in the locality of the plant collection have an impact on the chemical composition and bioactivity (**De Zoysa *et al*., 2019**) and the plants could exhibit better activity than the other reported areas. Thus, our work aimed to evaluate antibacterial activity of ethanol extracts from *C. macrostachyus* and *J. schimperiana* bark and leaf against certain bacterial pathogens such as *E. coli* O157 H:7, *S.* Typhi, *S. aureus* and *S. pyogenes.*

MATERIALS AND METHODS

Ethanol extract preparation

The leaves and bark of *C. macrostachyus* and *J. schimperiana* were collected from villages near Haramaya University, Ethiopia. Plant Taxonomists assisted in identification of the plants and the specimens were deposited in Haramaya University herbarium. The leaves and bark of the plants were dried and pulverized with the help of homogeniser. The leaf and bark powder (80 g each) were extracted with 400 ml of (1:5 w/v) 95% Ethanol. The extracts were evaporated to dryness at 40 ± 10^{o} C by rotary flash evaporator (Buchi type rotavapor) under reduced pressure. The crude ethanol extracts of leaf and bark were stored at 4^{0} C until further use.

Qualitative screening for phytochemicals

Qualitative analysis of ethanol extracts from *C. macrostachyus* and *J. schimperiana* bark and leaf was carried out to detect different phytochemicals viz. alkaloids, steroid, flavonoid, saponin, phlobatannins, tannin, terpenoids and phenolic compounds (**Harborne, 1984**).

Measurement of phytochemicals

Total phenolic content (TPC)

The total phenolic content (TPC) was measured by spectrophotometric method (**Amorim *et al*., 2012**). In brief, the ethanol extracts (500 µl) were added with Folin-Ciocalteu reagent (500 µl) and sodium carbonate solution (1 ml). Then, the final volume was adjusted to 10 ml by adding 8 ml of distilled water. The samples were kept at room temperature for 30 minutes and their absorptions were measured at 760 nm using distilled water as blank. The TPC was calculated as tannic acid equivalent (TAE) by the following equation:

$$TPC = C*V/M$$

Where, T is the TPC in mg/g of the extracts as Tannic Acid Equivalence (TAE), C is the concentration of tannic acid obtained from the calibration curve in mg/ml, V is the volume of the extract in ml and M is the weight of the extract used in g.

Terpenoid content

The terpenoid content was quantified following the method of (**Furguson, 1956**). The plant powder (2 g) was soaked with 97% ethanol (50 ml) for 4 hours. The extracts were filtered by Whatman No 1 filter paper and the filtrate was added into a separating funnel (250 ml) and then 50 ml of petroleum ether was added. The resultant mixture was shaken and allowed to stay for 5 minutes for layer formation. Then, the bottom layer was discarded while the top petroleum ether layer was collected and concentrated to dryness using rotary evaporator at 40 ºC for 18 hrs. The mass of dry extract, which was considered to represent crude terpenoids, was measured and its content was calculated as mg per the sample powder used.

Assays for antibacterial activity

Bacterial pathogens

For antibacterial activity study, two Gram negative pathogenic bacteria *Escherichia coli* O157 H:7 (ATCC 25922), *Salmonella* Typhi (ATCC 13311) and Gram-positive pathogenic bacteria *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 196151) were obtained from Ethiopian Public Health Institute (EPHI) and Ethiopian Biodiversity Institute (EBDI), Addis Ababa, Ethiopia. The guidelines of Clinical and Laboratory Standard Institute were employed to prepare the bacterial turbidity of the bacteria (**CLSI, 2012**).

Disc diffusion assay

The antibacterial effect of ethanol extracts of *C. macrostachyus* and *J. schimperiana* bark and leaf was determined by disc diffusion method against the bacterial pathogens *E. coli*, *S.* Typhi, *S. aureus* and *S. pyogenes* (**CLSI, 2012**). The bacterial strains were cultured in 25 ml nutrient agar plates prepared by transferring 25 ml of nutrient agar into a pre-sterilized Petri dish and allowed to solidify at room temperature. Muller Hinton agar (MHA) was used to smear 0.1ml suspension cultures of bacterial pathogens on plates. Sterile Whatman No. 1 paper discs (6 mm) were used to impregnate different concentrations (100 mg/ml, 125 mg/ml, 150 mg/ml) of ethanol extracts (0.1 ml/disc). The extract loaded discs were placed onto the agar surface at equidistance and the Petri dishes were incubated at 37°C for 24 hrs. Amoxicillin (25 µg/disc) was used as positive control. The experiment was performed in triplicates under sterile conditions. The antibacterial activity of the extracts was tested by measuring diameter of clear inhibition zones.

Determination of Minimum Inhibitory Concentration (MIC)

The ethanol extracts of *C. macrostachyus* and *J. schimperiana* bark and leaf were subjected for the minimum inhibitory concentrations (MIC) using broth dilution technique based on CLSI (2012). The concentrations of the extracts ranged from 100 mg/ml – 6.25 mg/ml and where 0.1 ml of each dilution were added to the different test tubes.

Statistical analysis

The experiments were performed in triplicate and results were presented as mean values ± Standard deviation (SD.) Differences between means were measured by one-way analysis of variance (ANOVA) using SPSS. *P*- values ≤ 0.05 were considered as showing significance difference.

RESULTS

Preliminary screening and Quantification

The results of preliminary phytochemical analysis are shown in Table 1. In both *C. macrostachyus* and *J. schimperiana*, ethanol extracts of both leaves and bark were found to show positive results for alkaloids, saponin, steroids, tannins and terpenoids. Flavonoids and phlobatannins were found absent in the screened plants. The quantification of phytochemicals in both the plants showed that ethanol extracts of *C. macrostachyus* leaves (3.82±0.82 mg/g Tannic acid equivalents) and bark (mg/g 3.01±0.07 Tannic acid equivalents) were found to contain significantly *(P* <0.05) higher TPC than that of ethanol extracts of *J. schimperiana* leaves (2.92±0.63 mg/g Tannic acid equivalents) and bark (mg/g 32. 14±0.13 mg/g Tannic acid equivalents). The ethanol leaf extract of *C. macrostachyus* significantly ($P < 0.05$) had the highest content of terpenoids (118.33±4.40 mg/g).

Table 1 Phytochemical screening from ethanol extracts from of *C. macrostachyus* and *J. schimperiana* leaves and bark

Phytochemicals	*Croton macrostachyus*		*Justicia schimperiana*	
	Leaf	Bark	Leaf	Bark
Alkaloids	+	+	+	+
Flavonoids	–	–	–	–
Saponin	+	+	+	+
Steroid	+	+	+	+
Tannin	+	+	+	+
Terpenoid	+	+	+	+
Phlobatannins	–	–	–	–

Legend: "+" indicates the presence of the phytochemicals while "–" indicates the absence of the phytochemicals.

Table 2 Quantitative analysis of phytochemicals in ethanol extracts of C. macrostachyus and J. schimperiana leaves and barks

Phytochemicals	Content (mg/g)			
	C. macrostachyus		*J. schimperiana*	
	Leaf	Bark	Leaf	Bark
Total Phenolics	$3.82±0.82^{A}$	$3.01±0.07^{B}$	$2.92±0.63^{A}$	$2.14±0.13^{B}$
Terpenoids	$118.33±4.40^{A}$	$103.33±4.40^{A}$	$90.00±2.88^{A}$	$86.67±1.67^{A}$

Legend: The values in the table show mean ± SEM (n=3). Superscripts in capital letters compare between means within row; and means with similar capital letters represent no significant difference whereas means with different capital letters are significantly different at $P< 0.05$.

Antibacterial activity against the pathogenic bacteria

Disc diffusion test

The effects of ethanol extracts of *C. macrostachyus* and *J. schimperiana* leaves and bark against the pathogenic bacteria were investigated and the results are exhibited (Table 3.). Our findings displayed that the plants' extracts possessed highest to moderate antibacterial activities against the tested bacteria. A significantly ($P< 0.05$) highest activity was shown by ethanol extract of *C. macrostachyus* leaf against the Gram-positive bacteria *S. aureus* with an inhibition of 16.17±0.67 mm at the concentration of 150 mg/ml.

Table 3 The antibacterial activities of the leaf and bark extracts of *C. macrostachyus* and *J. schimperiana* against human pathogenic bacteria

Plants	Conc. (mg/ml)	Plant Part	Inhibition zone (mm)			
			E. coli O157 H:7 (ATCC 25922)	*S.* Typhi (ATCC 13311)	*S. aureus* (ATCC 25923)	*S. pyogenes* (ATCC 196151)
C. macrostachyus	100	Leaf	$8.67±0.17^{Aa}$	$7.83±0.17^{Ab}$	$9.83±0.44^{Ba}$	$9.17±0.44^{Ab}$
		Bark	$8.00±0.29^{Aa}$	$7.50±0.29^{Ab}$	$9.50±0.29^{Ba}$	$8.40±0.21^{Ab}$
	125	Leaf	$12.17±0.17^{Aa}$	$11.17±0.72^{Ab}$	$14.43±0.23^{Ba}$	$11.47±0.24^{Ab}$
		Bark	$11.40±0.83^{Aa}$	$9.40±0.37^{Ab}$	$12.83±0.33^{Bb}$	$10.90±0.45^{Ab}$
	150	Leaf	$14.70±0.15^{Aa}$	$13.33±0.60^{Ab}$	$16.17±0.67^{Ba}$	$14.17±0.17^{Ab}$
		Bark	$12.83±0.93^{Aa}$	$12.37±0.75^{Ab}$	$14.50±0.29^{Ab}$	$13.77±0.15^{Ab}$
J. schimperiana	100	Leaf	$7.50±0.00^{Ab}$	$7.33±0.17^{Ab}$	$9.50±0.29^{Ba}$	$8.00±0.29^{Ab}$
		Bark	0	0	$8.50±0.29^{Aa}$	$7.67±0.17^{Bb}$
	125	Leaf	$9.83±0.16^{Aa}$	$9.70±0.15^{Bb}$	$13.67±0.17^{Aa}$	$10.33±0.33^{Bb}$
		Bark	$8.33±0.44^{Aa}$	$7.70±0.15^{Ab}$	$12.43±0.23^{Ba}$	$9.83±0.17^{Ab}$
	150	Leaf	$13.17±0.17^{Aa}$	$12.33±0.17^{Ab}$	$15.00±0.29^{Ba}$	$13.17±0.17^{Ab}$
		Bark	$11.83±0.93^{Aa}$	$11.77±0.93^{Ab}$	$14.17±0.17^{Aa}$	$12.33±0.17^{Ab}$
Amoxicillin	25 µg		18.00±.00	16±0.00	19±0.00	18.50±0.00

Legend: The values are Mean ± SEM (n=3). Superscripts in capital letters compare between means in row; and means with similar capital letters represent no significant difference, whereas means with different capital letters are significantly different at $P<0.05$. Small letter superscripts compare between means within a column; and means with similar small letters show no significant difference, whereas means with different small letters show significant difference at $P<0.05$.

Among the four bacterial strains tested, *S. aureus* was found more susceptible to the extracts at the concentration of 150 mg/ml of ethanol extracts of leaf and bark of both plants. Interestingly, at 100 mg/ml concentration, *J. schimperiana* showed no activity against the Gram-negative bacteria *E. coli* O157 H:7 and *S.* Typhi. The leaf and bark extracts of *C. macrostachyus* had highest antibacterial activity against *E. coli* and *S.* Typhi with the inhibition zones of 14.70±0.15 and 13.33±0.60 mm respectively at 150 mg/ml concentration. The positive control Amoxicillin displayed significantly higher activity against the tested bacterial pathogens than the plant extracts.

MIC values of the extracts

The values of MIC of the ethanol extracts of *C. macrostachyus* and *J. schimperiana* leaves and bark against the pathogenic bacteria are recorded in Table 4. The ranges of MIC values were 25±0.00 to 100±0.00 mg/ml. The leaf extract of *C. macrostachyus* exhibited the lowest MIC value (25±0.00) against *S. aureus* followed by the MIC value (41.67±8.33) of leaf extract of *C. macrostachyus* against *S. pyogenes*. Both Gram negative *E. coli* O157 H:7 and *S. Typhi* were observed to be more resistant to the ethanol extracts of the plant parts.

Table 4 The minimum inhibitory concentration (mg/ml) of the leaf and bark ethanolic crude extracts (0.1ml) of C. macrostachyus and J. schimperiana against the bacterial species

Bacterial strains	**MIC (mg/ml)**			
	C. macrostachyus		***J. schimperiana***	
	Leaf extract	Bark extract	Leaf extract	Bark extract
***E. coli* O157 H:7 (ATCC 25922)**	50±0.00	66.66±16.67	100±0.00	0
***S.* Typhi (ATCC 13311)**	66.66±16.67	83.33±16.67	100±0.00	0
***S. aureus* (ATCC 25923)**	25±0.00	50.00±0.00	66.67±16.67	83.33±16.67
***S. pyogenes* (ATCC 196151)**	41.67±8.33	66.67±16.67	66.67±16.67	100±0.00

Legend: Values are expressed as Mean ± SEM (n=3)

DISCUSSION

The present investigation reported qualitative phytochemical analysis, quantification of total phenolics and terpenoids and antibacterial activity ethanol extracts from *C. macrostachyus* and *J. schimperiana* leaves and bark against the pathogenic bacteria include Gram negative *E. coli* O157 H:7 and *S. Typhi* and Gram-positive *S. aureus* and *S. pyogenes*. With regard to qualitative phytochemical analysis, extracts of both plants contain alkaloids, saponin, steroids, tannins and terpenoids. Our results are in covenant with the previously published reports in the case of *C. macrostachyus* (**Kibret *et al.*, 2018**) and for *J. schimperiana* (**Mekonnen *et al.*, 2018**). The quantification of phytochemicals revealed that C. *macrostachyus* contained high TPC and terpenoid contents which could be attributed to its antibacterial potential. The phenolic terpenoid compound has been proved to disturb cell membrane integrity and ion leakage of gram positive and gram-negative bacteria (**Erguden, 2021**). The evaluation of antibacterial activity of the studied plants revealed a strong activity against the tested pathogens. The antibacterial activities of the ethanol extracts from leaf and bark of the investigated plants were found inferior than the standard control Amoxicillin which is due to the crude nature of the plant extracts (**Mengiste *et al.*, 2015**). Concerning to *C. macrostachyus*, the ethanol extract of the leaves and bark displayed strong antibacterial activity against the gram-positive bacteria. Regarding *J. schimperiana*, the antibacterial activity of the ethanol leaf extract was found stronger against the gram-positive bacteria. Surprisingly, ethanol extract from *J. schimperiana* bark failed to exhibit any antibacterial activity against *E. coli* O157 H:7 and *S.* Typhi. The stronger antibacterial activity of ethanol extracts both the plants against gram positive bacteria *S. aureus* and *S. pyogenes* than the gram-negative bacteria such as *E. coli* O157 H:7 and *S.* Typhi was due to cell wall structure of the gram-positive bacteria with thick layer of peptidoglycan sheets in the outer wall (**Kitonde *et al.*, 2013**). However, gram positive bacteria have been posing threat to humans as they are developing resistance to antibacterial drugs. For instance, *Streptococcus pyogenes* has been known to cause pharyngitis among the paediatric group and infection of skin and soft tissue which is resistant to erythromycin and clindamycin (**Kebede *et al.*, 2021**). Similarly, *S. aureus* cause soft tissue infections. bacteraemia, and fatal pneumonia and this bacterium is resistant to methicillin (**Guo *et al.*, 2020**). The gram-negative bacteria *S.* Typhi, causative agent of typhoid disease, has become resistant to chloramphenicol and ampicillin (**Dyson *et al.*, 2019**). The enterohemorrhagic *E. coli* O157 H:7 causes food borne and water borne diseases like bloody or non-bloody diarrhea in humans (**Dean-Nystrom *et al.*, 1997**) and this strain was reported to show resistant against antibiotics (**Ferdous *et al.*, 2016**). Furthermore, the increasing side effects caused by the synthetic antibacterial agents jeopardizes the human health (**Mohsen *et al.*, 2020**). To overcome above mentioned issues, development of alternative drugs is essential. Thus, the current study was conducted. In our study, the ethanol extracts of *C. macrostachyus* and *J. schimperiana* leaves and bark displayed a potent activity against these bacteria with good inhibition zone and least MIC value. Hence, further studies are warranted to isolate phenolics and terpenoids using bioactivity-directed fractionation from the currently studied plants to combat drug resistant pathogenic bacteria.

CONCLUSION

The findings of the study revealed that ethanol extract of *C. macrostachyus* and *J. schimperiana* leaves and bark showed significant antibacterial effects against Gram negative *E. coli* O157 H:7 and *S.* Typhi and Gram-positive *S. aureus* and *S. pyogenes*. Gram negative bacteria were more resistant than the gram-positive bacteria. *E. coli* O157 H:7 and *S.* Typhi were found to show strong resistance to plant extract at low concentrations. Among the pathogens, *S. aureus* was found to me most sensitive to the plant extracts. The antibacterial properties of the two plants against the tested pathogenic bacteria could be attributed to the total phenolic and terpenoids contents. Further in vivo antibacterial activity and isolation of active components by bioactivity-guided fractionation is vital.

Acknowledgements: The authors express their deep sense of gratitude to School of Biological Sciences and Biotechnology for providing facilities to perform this research.

REFERENCES

ABEBE, W., ZHANG, W., ZHANG, S., XIE, G. 2018. Chemical composition and antimicrobial activity of essential oil from *Justicia schimperiana. Journal of Pharmacognosy and Natural Products*, 4, 154. https://doi.org/10.4172/2472-0992.1000154

ALI SHAH, S., NADEEM, M., SYED, S., et al. 2020. Antimicrobial sensitivity pattern of *Salmonella* Typhi: Emergence of resistant strains. *Cureus* 12(11), e11778. http://dx.doi.org/10.7759/cureus.11778 .

AMORIM, K., LAGE-YUSTY M., LÓPEZ-HERNÁNDEZ, J. 2012. Changes in bioactive compounds content and antioxidant activity of seaweed after cooking processing, *CyTA - Journal of Food*, 10(4), 321-324. https://doi.org/10.1080/19476337.2012.658871

ANTIMICROBIAL RESISTANCE COLLABORATORS, 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*, 399, 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

CLSI, 2012. *Performance Standard for antimicrobial disc susceptibility tests: Approved Standard, 11th edition*. CLSI document M02-A11.32:1. Clinical Laboratory Standard Institute, Wayne, PA.950 West Valley Road, Suite 2500 Wayne, PA 19087 USA.

DEAN-NYSTROM, E. A., BOSWORTH, B.T., CRAY, W.C., MOON H. W. 1997. Pathogenicity of *Escherichia coli* O157:H7 in the Intestines of Neonatal Calves. *Infection and Immunity*, 65(5), 1842–1848. https://doi.org/10.1128/iai.65.5.1842-1848.1997

DE ZOYSA, M.H.N., RATHNAYAKE, H., HEWAWASAM, P. WIJAYARATNE, W.M.D.G.B. 2019. Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. International Journal of Microbiology Article ID 7431439, https://doi.org/10.1155/2019/7431439

DYSON, Z.A., KLEMM, E. K., PALMER, S., DOUGAN, G. 2019. Antibiotic Resistance and Typhoid. *Clinical Infectious Diseases*. 68, S165-S170. https://doi.org/10.1093/cid/ciy1111

ERGÜDEN, B. 2021. Phenol group of terpenoids is crucial for antibacterial activity upon ion leakage. *Letters in Applied Microbiology*, 73(4), 438-445. https://doi.org/10.1111/lam.13529

FERDOUS, M., KOOISTRA-SMID, A.M.D., ZHOU, K., ROSSEN, J.W.A. FRIEDRICH, A.W. 2016. Virulence, Antimicrobial Resistance Properties and Phylogenetic Background of Non-H7 Enteropathogenic *Escherichia coli* O157. *Frontiers in Microbiology*, 7, 1540. https://doi.org/10.3389/fmicb.2016.01540

FERGUSON, N. M. 1956. A Text book of Pharmacognosy. Mac Milan Company, New Delhi. p. 191.

FOSTER, T. J. 2017. Antibiotic resistance in *Staphylococcus aureus*. Current status and future prospects. *FEMS Microbiology Reviews*, 41, 430–449. https://doi.org/10.1093/femsre/fux007

GEYID, A., ABEBE, D., DEBELLA, A. et al. 2005. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *Journal of Ethnopharmacology*, 97, 421–427. https://doi.org/10.1016/j.jep.2004.08.021

GIDAY, M., TEKLEHAYMANOT, T., ANIMUT, A., MEKONNEN, Y. 2007. Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. *Journal of Ethnopharmacol*ogy, 110, 516-525. https://doi.org/10.1016/j.jep.2006.10.011

GUO, Y., SONG, G., SUN, M., WANG, J. WANG, Y. 2020. Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. *Frontiers in Cell and Infectious Microbiology*. 10, 107. https://doi.org/10.3389/fcimb.2020.00107

HABTAMU, A., MULATU, O. TSDEKE, L. 2014. Traditional medicinal plants utilization, management and threats in Hadiya zone. *Journal of Medicinal Plant Studies*, 2, 94-108

HARBORNE, J. B. 1984. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. ISBN: 978-94-009-5570-7. Chapman and Hall Ltd. London.

HASSAN, H. M., JIANG, Z.-H., ASMUSSEN, C., MCDONALD, E. ET QIN, W. 2014. Antibacterial activity of northern Ontario medicinal plant extracts. *Canadian Journal of Plant Sciences*, 94, 417-424. https://doi.org/10.4141/CJPS2013-258

ISUNU, L. E., OMOYA, F. O., OGUNDARE, A. O., BABATUNDE, O. J., BAYODE, M. T., AJAYI, K. O. 2022. Antibacterial activity of *Andrographis paniculata* (Burm. F.) methanol leaf extract on bacteria consortia isolated from blood of diabetic patients. *Bacterial Empire*, 11(5), e381. https://doi.org/10.36547/be.381

KEBEDE, D., ADMAS, A., MEKONNEN, D. 2021. Prevalence and antibiotics susceptibility profiles of *Streptococcus pyogenes* among paediatric patients with acute pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Microbiology*. 21, 135. https://doi.org/10.1186/s12866-021-02196-0

KIBRET, B., ALEMAYEHU, I., ENDALE, M., ASEGU, D. 2018. Comparative Study of the Antibacterial Activity of Leaves of *Croton macrostachyus* and *Aloe Vera*. *Advances in Life Science and Technology*. 54, 22-28.

KITONDE, C. K., FIDAHUSEIN D. S., LUKHOBA C.W., JUMBA, M.M. 2013. Antimicrobial activity and phytochemical study of *Vernonia Glabra* (Steetz) Oliv. & Hiern. in Kenya. *African Journal of Traditional Complementary and Alternative Medicine*, 10(1), 149–157. https://doi.org/10.4314/ajtcam.v10i1.20

MECHESSO, A. F., AYELE, T., RETA, T., WONDEMAGEGN, E., TADESSE, E. 2016. Experimental evaluation of wound healing activity of *Croton macrostachyus* in rat. *African Journal of Pharmacy and Pharmacology*, 10(39), 832-838. https://doi.org/10.5897/AJPP2015.4454

MEKONNEN, B., ASRIE A. B., WUBNEH Z. B. 2018, Antidiarrheal Activity of 80% Methanolic Leaf Extract of *Justicia schimperiana*. *Evidence-Based Complementary and Alternative Medicine*. Article ID 3037120, https://doi.org/10.1155/2018/3037120

MESFIN, T., ABERRA, G. ASFAW, D. 2010. *In vitro* anti-Neisseria gonorrhoeae activity of *Albizia gummifera* and *Croton macrostachyus*. *Revista Cenic Ciencias Biologicas*, 41(4), 1-11.

MOHSEN, S., DICKINSON, J. A., SOMAYAJI, A. 2020. Update on the adverse effects of antimicrobial therapies in community practice. *Canadian Family Physician*. 66, 651-659.

OBEY, J. K., VON WRIGHT, A., ORJALA, J., KAUHANEN J.. TIKKANEN-KAUKANEN C. 2016. Antimicrobial activity of *Croton macrostachyus* stem bark extracts against several human pathogenic bacteria. *Journal of Pathogens*, Article ID 1453428. http://dx.doi.org/10.1155/2016/1453428

PIERETTI, B. CANOVARI, B. MORETTI, M. PIERETTI, C. PAZZAGLIA, E. 2017. Drug-resistant *Streptococcus pyogenes*: a case report of pyoderma and Cellulitis. *Microbiologia Medica.* http://dx.doi.org/10.4081/mm.2017.6617

SASIKUMAR, J.M., DOSS, P.A., DOSS, A. 2005. Antibacterial activity of *Eupatorium glandulosum* leaves. *Fitoterapia*. 76, 240–243. http://dx.doi.org/10.1016/j.fitote.2004.12.001

SINGLETON, V. L., ORTHOFER, R., LAMUELA-RAVENTOS, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299,152-178. http://dx.doi.org/10.1016/s0076-6879(99)99017-1

TALA, M.F., TAN, N.H., NDONTSA, B.L., TANE, P. 2013. Triterpenoids and phenolic compounds from *Croton macrostachyus*. Biochem Syst Ecol 51,138–141. http://dx.doi.org/10.1016/j.bse.2013.08.001

TAMRAT, T., MELKAMU, B. DESALEGN, A. 2015. Antibacterial and antagonistic activity of selected traditional medicinal plants and herbs from East Wollega zone against clinical isolated human pathogens. *Science, Technology and Arts Research Journals*, 4(3), 175-179.

TENE, NDONTSA B. L., TANE, P., TAMOKOU, J. D. KUIATE, J. 2009. Antimicrobial diterpenoids and triterpenoids from the stem bark of *Croton macrostachys* Mathieu Int. J. Biol. Chem. Sci. 3(3), 538-544. https://doi.org/10.4314/ijbcs.v3i3.45331

TEKLEHAYMANOT, T. GIDAY, M. 2007. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia, *Journal of Ethnobiology and Ethnomedicine*, 3, 12. https://doi.org/10.1186/1746-4269-3-12

TREASE, G. E. EVANS, W.C. 1989. Pharmacognosy. 11th (edn). Brailliar Tiridel Can. Macmillian Publishers.

TSURUGA, M. NAKAJIMA, H. MAGAE, J. 2007. Immunosuppressive activity of4-O-Methylascochlorin, *The Journal of Antibiotics*, 60, 20–26. https://doi.org/10.1038/ja.2007.3

UMER, S ASRES K., VEERESHAM, C. 2010. Hepatoprotective activities of two Ethiopian medicinal plants, *Pharmaceutical Biology*, 48(4), 461-468, https://doi.org/10.3109/13880200903173593

WAGATE, C. G. MBARIA, J. M. GAKUYA D. W. et al., 2010. Screening of some Kenyan medicinal plants for antibacterial activity. *Phytotherapy Research*, 24(1), 150–153, https://doi.org/10.1002/ptr.2866

YONG, Y., TESSO, H., TERFA, A. DEKEBO, A., DINKU, W., LEE, Y. H . SHIN, S. Y.. LIM, Y. 2017. Biological evaluation of the diterpenes from *Croton macrostachyus*. *Applied Biological Chemistry*, 60**,** 615–621. https://doi.org/10.1007/s13765-017-0317-5

ZEWDITU S., SASIKUMAR J.M., KEBEDE A., TEJU, E. 2022. Antibacterial effects of extracts from *Tanacetum parthenium* L. leaves. *Bacterial Empire*. 5(1): e394. https://doi.org/10.36547/be.394