ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2024; 12(1): 121-126
© 2024 JMPS
Received: 19-11-2023
Accepted: 22-12-2023

Steward Mudenda
Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, Zambia

Ronald Chalwe
PACT, USAID - Zambia Integrated Health Project, North Western provincial HUB, Solwezi, Zambia

Reagan Kabuka
Department of Pharmacy, School of Health Sciences, Levy Mwanawasa Medical University, Lusaka, Zambia

Billy Chabalenge
Department of Medicines Control, Zambia Medicines Regulatory Authority, Lusaka, Zambia

Corresponding Author:
Steward Mudenda
Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, Zambia

Antibacterial activity of *Cassia abbreviata* oliv roots against *Neisseria gonorrhoeae*: A potential traditional medicine for the treatment of sexually transmitted infections

Steward Mudenda, Ronald Chalwe, Reagan Kabuka and Billy Chabalenge

DOI: https://doi.org/10.22271/plants.2024.v12.i1b.1632

Abstract
Traditional medicines are useful in the management of various diseases, especially in low- and middle-income countries. This study analysed the antibacterial activity of *Cassia abbreviata Oliv* roots against *Neisseria gonorrhoeae*. This was a laboratory-based study in which the extracts of *Cassia abbreviata Oliv* roots were tested against *Neisseria gonorrhoeae*. Additionally, the extracts of *Cassia abbreviata Oliv* roots were combined with tetracycline to determine their synergistic effect. Data were analysed using IBM SPSS version 22.0. All extracts were active against *Neisseria gonorrhoeae,* with a minimum inhibitory concentration of 125 µg/ml for the ethanol and 62.5 µg/ml for the aqueous extract. Despite both extracts having antibacterial activity against *Neisseria gonorrhoeae,* their effect were lower than that of tetracycline. There was an increase in activity when tetracycline was combined with aqueous extract. This study demonstrated that traditional medicines should be studied further for use in drug discovery, especially with increasing resistance to conventional antibiotics.

Keywords: Antibacterial activity, antimicrobial resistance, *Cassia abbreviate,* Gonorrhoea, *Neisseria gonorrhoeae,* traditional medicine, Zambia

Introduction

Gonorrhea is a global public health problem that requires immediate international public health resources and attention [1]. It is a purulent infection of mucous membrane surfaces caused by the pathogen *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and responsible for nearly 86.9 million new infections each year [2]. The bacterium principally infects the urethra in men and the endocervix in women [3].

The treatment of gonorrhoea is complicated by the global emergence and dissemination of strains of *N. gonorrhoeae* that are resistant to most antimicrobial agents available for treatment [1]. *N. gonorrhoeae* has developed resistance to antibiotics previously recommended for first-line empirical treatment of gonorrhoea [4]. Resistance has been reported to antibiotics including ciprofloxacin, azithromycin, cefixime, and ceftriaxone [4-8]. Further, resistance to ceftriaxone, the last option for monotherapy, is evolving [9]. Consequently, antimicrobial resistance (AMR) is a global public health problem, with consequences including increased morbidity and mortality, and increased medical costs [10-16].

Accurate detection of drug-resistant pathogens is essential for the appropriate management of gonorrhoea and the prevention of the spread of infections as well as complications in individual patients [17]. The World Health Organization's (WHO's) global strategy for the prevention and control of Sexually Transmitted Infections (STIs) stipulates that comprehensive STI management should include as a minimum, accurate diagnosis by syndrome or laboratory diagnosis, plus effective treatment to prevent complications and further transmission [18-20].

Traditional medicines have been reported to be effective against various microorganisms [21]. For this reason, they can be used to treat various infectious diseases. Consequently, the use of traditional medicines has increased due to the growing problem of AMR to conventional medicines.

To this effect, there is a need for intensified research to identify novel antimicrobial compounds from natural products [22-24].

Cassia abbreviata (C. abbreviata) is one of the plants with vast medicinal values [25, 26]. It is a small, umbrella-shaped deciduous shrub in the genus *Cassia* that belongs to the family *Caesalpiniaceae* [25]. The shrub grows between 2 and 15 m in height, with a medium-round canopy [25]. It has a dark grey to brown stem bark that is very rough on older trees, as well as young branchlets that are glabrous, pubescent, or puberulous [27]. Some studies have reported the potential use of the plant treatment of gonorrhoea [28], increased sperm production [29], and potential use in lung cancer and treatment of diabetes [30, 31].

In Zambia, *C. abbreviata* is referred to as Umunsokansoka by the Bemba-speaking people, Matholisi or Mulesa by the Chewa-speaking people, and as Mululwe by the Ila-speaking people [24]. Traditionally, the roots are dried, dissolved in water and taken orally to treat sexually transmitted infections such as gonorrhoea, as well as alleviate stomachaches and symptoms associated with pre-menstrual syndrome [24]. The stem bark is also crushed, soaked in water taken orally to treat diarrhoea, and applied to help treat abscesses and many other types of skin lesions [24].

C. abbreviata grown in Zambia is a rich source of phytochemicals including tannins, flavonoids, glycosides, saponins, alkaloids, triterpenoids, and sterols which are thought to be responsible for its therapeutic indications such as gonorrhoea [24]. Studies have shown the antibacterial activity of *C. abbreviata* against specific pathogens [24, 32, 33]. The ethanolic extract, for instance, was found to have antibacterial activity against *Staphylococcus aureus* with a minimum inhibitory concentration of 5mg/ml [32]. However, there is little information about its activity against *N. gonorrhoeae* for the plant grown in Zambia. Therefore, this study was undertaken to investigate the *in-vitro* antibacterial activity of *Cassia abbreviata Oliv* root extract against *Neisseria gonorrhoeae*.

Materials and Methods

Study design and site

This was a laboratory-based study in which *N. gonorrhoeae* bacteria isolated from a patient and cultured in the laboratory was used to investigate the antimicrobial activity of *C, abbreviata* and the synergistic effect of *C. abbreviata* and tetracycline in the treatment of gonorrhoea *in-vitro*. This study was carried out at the University Teaching Hospital (UTH), Food and Drugs Control Laboratory in the month of September 2020. Only plants with green fresh leaves were collected.

Materials

Neisseria gonorrhoeae strain (ATCC 49226), bacterial culture plates, swabs, paper discs, agar plates, ethanol, distilled water and *C. abbreviata Oliv* plant roots were the materials used in this study. Ethanol and distilled water were used as solvents to make extracts of the plant. *C. abbreviata Oliv* roots were collected in August 2020 from Kapiri Mposhi, Central province of Zambia. The plant parts were taken to Mount Makulu Research Centre in Chilanga in the Herbarium section for botanical identification and authentication of the plants.

The roots were separated from the stem using an axe, washed and cleaned to remove insects and foreign matter. The *C. abbreviata oliv* roots were then cut into smaller pieces using a knife, spread on a newspaper and left to dry in the shade for 21 days. The dried roots were then prepared into a powder using a mortar and pestle. The powdered roots were stored in amber-coloured containers [34].

The bacteria used in this study were obtained from the University Teaching Hospital in Lusaka, Zambia.

Preparation of the aqueous and organic extract

The maceration method was used to extract the active ingredients from the plant. The ethanol extract was prepared by mixing 60 grams of powdered root with 200 ml ethanol (95%) in a conical flask and subjected to mechanical shaking for 8 hours. The sample was then filtered using Whatman number one filter paper and the filtrate was then evaporated at 60 ºC using a rotary evaporator under reduced pressure. The remaining dry residue was labelled ET and stored in an airtight container until the time of the experiment.

The aqueous extract was prepared using distilled water. A weight of 50 grams of the powdered root was mixed with 150 ml of distilled water and filtered on the Whatman number one filter paper. The extract was labelled AQ and stored in the refrigerator at a temperature of 2 to 8 ºC [34]. The extracts obtained were then reconstituted with Dimethyl Sulphoxide (DMSO) to determine their antibacterial activity.

The percentage yield was calculated using the formula below.
Percentage

$$\text{Extractive yield}\left(\frac{w}{w}\right) = \frac{\text{Weight of extract recovered}}{\text{Starting weight of dry plant}} \text{ X } 100$$

Inoculum preparation and inoculation

The direct colony suspension method was used. The bacteria inoculum suspension was prepared using sterile saline and turbidity was compared with 0.5 McFarland turbidity standards. The media was inoculated by swabbing the agar surface in two directions at 90º angle to each other and the third line at 45º angle. Swabbing was then done to the rim of the agar. The plates were allowed to stand for 24 hours to allow excess inoculum to be absorbed before the test plant was applied.

Determination of Antibacterial Activity

Paper disk diffusion assay

The suspensions of testing microorganisms were spread on Muller Hinton Agar (MHA) medium, and the plates were labelled. The filter paper discs (6 mm in diameter) were placed in reconstituted (with DMSO) extracts of different concentrations and were allowed to stand for 30 minutes to let the disc soak the extract. The soaked discs were then put on agar plates which were inoculated with the microorganism. The plates were subsequently incubated at 37 ºC for 24 hours. After incubation, the growth inhibition zones were measured using the diameter of the zone of inhibition in millimetres, similar to what was reported in another study [35]. The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration at which a clear zone of microbial growth inhibition was observed.

Synergism between plant extract and tetracycline

The antibiotic disk (diameter=6 mm) was placed in each test extract for 30 minutes and later put on the surface of each inoculated plate to identify synergistic effects between the plant extract and antibiotic. The plates were then incubated at 37 ºC and the zone of inhibition was measured after 24 hours of incubation. MIC was also determined as the lowest concentration at which a clear zone of microbial growth inhibition was observed.

Data analysis

Data analysis was done using IBM Statistical Package for Social Sciences (SPSS) version 22.0. GraphPad prism statistical software version 7.0 was used to compare the mean inhibition zone produced by the different concentrations of organic (ethanol) *C. abbreviata* extract on *N. gonorrhoeae* to the standard drug (tetracycline) as well as compared the standard drug to a mixture of standard drug and ethanol extract (at 1000 µg/ml concentration) using Repeated Measures ANOVA.

Ethical approval

Ethical approval was obtained from the University of Zambia Health Sciences Research Ethics Committee (UNZAHSREC). Additionally, permission was obtained from the Food and Drug Control Laboratory and University Teaching Hospital to conduct the study.

Results

This study found that the percentage yields of the ethanol and water extracts were 19.5% and 14.4%, respectively (Figure 1).

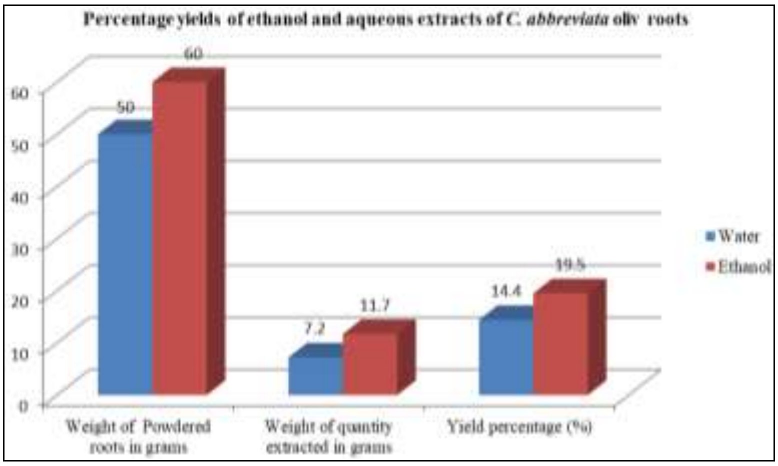

Fig 1: Showing percentage yields of ethanol and water extracts of *C. abbreviata oliv* roots.

The concentrations of ethanol and aqueous of *C. abbreviata* and the associated zones of inhibition (Table 1).

Table 1: Antibacterial activity of the organic (ethanol) and aqueous (water) extracts of *Cassia abbreviata* oliv roots showing the mean of zones of inhibition.

Ethanol	Concentration	Zone of inhibition			Mean
	125 µg/ml	4.5	4.23	4.6	4.4
	250 µg/ml	10	9.8	10.2	10
	500 µg/ml	12	11.8	12.1	12
	1000 µg/ml	12.5	12.3	12.7	12.5
	Standard	18	18.1	17.9	18
	Standard + sample (1000µg/ml)	17.5	17.2	17.6	17.4
Aqueous	62.5 µg/ml	3.5	3.1	3.6	3.4
	125 µg/ml	6.5	6.3	6.6	6.5
	250 µg/ml	10.5	10.2	10.7	10.5
	500 µg/ml	12	12.3	11.9	12.1
	1000 µg/ml	14	14.2	13.8	14
	Standard	18	18.1	17.9	18
	Standard + sample (1000 µg/ml)	25	24.8	25.2	25

The concentrations (mg/ml) of the organic (ethanol) extract of *C. abbreviata oliv* roots had dose-dependent activity against *N. gonorrhoeae* which was lower than that of tetracycline (Table 2). DMSO did not produce antibacterial activity (Table 2).

Table 2: The mean and standard deviations in the size of the inhibition zones produced by different concentrations of organic (ethanol) extract of *C. abbreviata oliv roots*

Ethanol	Concentration	Zone of inhibition			Mean ± SD of the inhibition zones
	DMSO	0	0	0	0
	125 µg/ml	4.5	4.23	4.6	4.40±0.19
	250 µg/ml	10	9.8	10.2	10.0±0.20
	500 µg/ml	12	11.8	12.1	12.0±0.15
	1000 µg/ml	12.5	12.3	12.7	12.5±0.20
	Standard	18	18.1	17.9	18.0±0.10
	Standard + sample (1000 µg/ml)	17.5	17.2	17.6	17.4±0.21

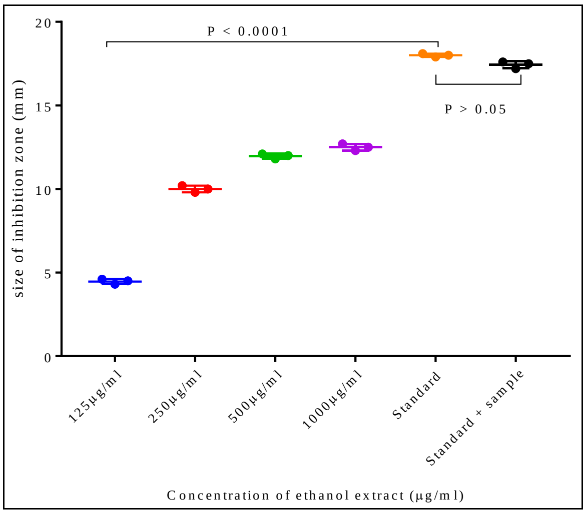

Fig 2: Zones of inhibition of ethanol extracts of *C. abbreviata oliv* roots corresponding to their respective concentrations.

The minimum inhibitory concentration (MIC) for the ethanol extract was found to be 125 µg/ml as shown in Figure 2. There was a significant difference observed in the inhibition activity of the ethanol extract and that of tetracycline. The Tetracycline standard had high antibacterial activity compared to the ethanol extract and the mixture of tetracycline and ethanolic extract (Figure 2).

The aqueous (water) extract of *C. abbreviata oliv* roots had variable antibacterial activity against *N. gonorrhoeae* (Table 3). The antibacterial activity of the aqueous extract was lower than that of the tetracycline standard and no activity was noted for the DMSO.

Table 3: The mean and standard deviations in the size of the inhibition zones produced by different concentrations of aqueous (water) extract of *C. abbreviata oliv roots.*

Aqueous Concentration	Zone of inhibition Mean ± SD			
DMSO	0	0	0	0
62.5 µg/ml	3.5	3.1	3.6	3.4±0.26
125 µg/ml	6.5	6.3	6.6	6.5±0.15
250 µg/ml	10.5	10.2	10.7	10.5±0.25
500 µg/ml	12	12.3	11.9	12.1±0.21
1000 µg/ml	14	14.2	13.8	14.0±0.20
Standard	18	18.1	17.9	18.0±0.10
Standard + sample (1000 µg/ml)	25	24.8	25.2	25.0±0.20

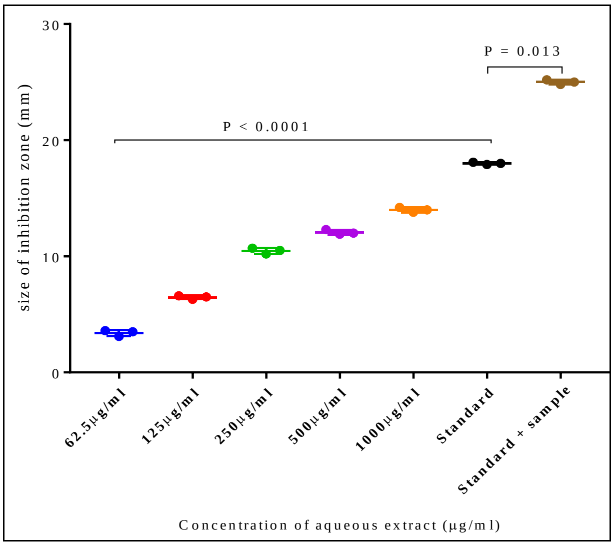

Fig 3: Zones of inhibition of water extracts of *Cassia abbreviata oliv* roots corresponding to their respective concentrations.

The MIC for the aqueous extract was found to be 62.5 µg/ml as shown in Figure 3. There was a significant difference observed in the inhibition activity between the different concentrations of the aqueous extract, tetracycline, and that of the mixture of tetracycline and aqueous extract. Tetracycline standard exhibited higher antibacterial activity than the aqueous extract. Consequently, a mixture of tetracycline and the aqueous extract had greater antibacterial activity compared to the standard tetracycline.

Discussions

This study investigated the antibacterial activity of *C. abbreviata* olive roots against *N. gonorrhoeae.* The present study yielded an ethanol extract of 19.5% and an aqueous extract of 14.4%. We found that *C. abbreviata* had antibacterial activity against *N. gonorrhoeae* and the effect was higher for the aqueous extract compared to the ethanol extract.

The wide traditional use of *C. abbreviata oliv* roots in the treatment of STIs including gonorrhoea is an indication that the roots of this plant have antibacterial activity against *N. gonorrhoeae.* Optimisation of *C. abbreviata* use can potentially help in dealing with the bacterial resistance that has emerged to antibiotics in recent years [36].

The present study revealed that the ethanol extract of *C. abbreviata* had a higher percentage yield of 19.5% while aqueous extract had a percentage yield of 14.4%. These results are similar to the ones obtained in a study which was done in Zambia at the University of Zambia using the bark of *Cassia abbreviata oliv* extracts which had ethanol and aqueous percentage yields of 19% and 12% respectively [34]. This could mean that most of the phytochemicals were more soluble in ethanol than water.

Our study found that both extracts (aqueous and ethanol) of *C. abbreviata oliv* roots showed *in-vitro* antibacterial activity against *N. gonorrhoeae.* The observed MICs were 125 µg/ml and 62.5 µg/ml for the ethanol and aqueous extracts, respectively, indicating that the aqueous extract had higher activity than the ethanol extract. The results of this research, therefore, support the use of an aqueous decoction of *C. abbreviata oliv* roots in the treatment of gonorrhoea, as reported earlier [3, 37].

According to Mulubwa (2015), the MICs of organic and aqueous extracts of *C. abbreviata oliv* bark were determined to be 46.88 µg/ml and 93.75 µg/ml respectively [34]. However, the *C. abbreviata* plant for this study was cultivated in Lusaka. Therefore, the difference could be a result of differences in the location of the plant, weather pattern, and type of soil between Kapiri Mposhi and Lusaka. Another possible reason for the difference could be due to differences in the quantity of phytochemicals in the extracts.

The difference observed in MICs could arise due to differences in the extraction of certain phytochemicals. Some phytochemicals were more soluble in a particular solvent and these phytochemicals may be responsible for antibacterial activity against *N. gonorrhoeae.* The activity of plant extract is said to be better when the MIC is lower or equal to 100 µg/ml [38], therefore, our findings indicated that the aqueous extract had better antibacterial activity than ethanol extract in the treatment of gonorrhoea. Studies have shown that the antibacterial activities of *C. abbreviata* are due to its phytochemical constituents belonging to different chemical classes including anthraquinones, phenolics, triterpenoids, organic acids, and proanthocyanidins [24, 26]. The polyphenolic nature of proanthocyanidins and their structural diversity make them potential targets for the discovery of new antimicrobial agents [26].

The present study demonstrated that the different concentrations of the ethanol extracts produced less antibacterial activity compared to tetracycline. Conversely, tetracycline produced slightly higher zones of inhibition compared to a combination of tetracycline and 1000µg/ml of the ethanol extract. Therefore, our findings show that the use of ethanol extracts of *C. abbreviata oliv* roots together with tetracycline did not produce a synergism as the effect of tetracycline seemed to have reduced.

Furthermore, our study also found that the aqueous extract produced less antibacterial activity compared to tetracycline. However, tetracycline alone produced smaller zones of inhibition compared to the mixture of tetracycline and 1000 µg/ml of the aqueous extract. Therefore, a combination of tetracycline (standard drug) and 1000 µg/ml of aqueous extract produced a significant synergism.

With the rise in resistance to conventional therapeutic drugs, the utilization of herbal and traditional remedies sometimes in combination with other medications has increased tremendously over the last decade [39, 40]. Therefore, our study supports the findings of Ritchie in 2007 [39], which demonstrated that the combination of herbal or traditional remedies with conventional medicines could produce a better effect.

We need to include something on the limitations of the study. One of them could be the characterisation of the active constituent causing the activity was not done.

Conclusion

In conclusion, there were variations in the antibacterial activity of the ethanol and aqueous extract of *Cassia abbreviata Oliv* roots on *Neisseria gonorrhoeae*. The aqueous extract produces better antibacterial activity than the ethanol extract on *Neisseria gonorrhoeae*. There was an increase in activity when tetracycline was combined with aqueous extract. Therefore, this study supports the combination of tetracycline and aqueous extract in the treatment of gonorrhoea. However, further studies are required on specific phytochemicals of *Cassia abbreviata Oliv* roots to come up with a potent antibiotic for the treatment of gonorrhoea.

Recommendations

Phytochemical screening is the first step in realizing the potential of plants that are used in the traditional system of medicines. Further studies should therefore be carried out, to extract or separate these pure metabolites that have potential antibacterial activity against *Neisseria gonorrhoeae* and test them to observe their effects *In vitro* and in vivo.

References

1. Unemo M, Golparian D, Shafer WM. Challenges with gonorrhoea in the era of multi-drug and extensively drug resistance-are we on the right track? Expert Review of Anti-Infective Therapy. 2014;12(6):653-656. doi:10.1586/14787210.2014.906902
2. Unemo M, Seifert HS, Hook EW, Hawkes S, Ndowa F, Dillon JAR. Gonorrhoea. Nat Rev Dis Prim. 2019;5:1-23. doi:10.1038/s41572-019-0128-6
3. Otto RBD, Ameso S, Onegi B. Assessment of antibacterial activity of crude leaf and root extracts of Cassia alata against Neisseria gonorrhoea. Afr Health Sci. 2014;14(4):840-848. doi:10.4314/ahs.v14i4.11
4. Unemo M, Lahra MM, Escher M, Eremin S, Cole MJ, Galarza P, *et al*. WHO Global Antimicrobial resistance surveillance for *Neisseria gonorrhoeae* 2017-18: a retrospective observational study. The Lancet Microbe. 2021;2(12):e627-e636. doi:10.1016/S2666-5247(21)00171-3
5. Li X, Le W, Lou X, Wang B, Genco CA, Rice PA, *et al*. *In vitro* Efficacy of Gentamicin Alone and in Combination with Ceftriaxone, Ertapenem, and Azithromycin against Multidrug-Resistant *Neisseria gonorrhoeae*. Microbiol Spectr. 2021;9(1):e00181-21. doi:10.1128/spectrum.00181-21
6. Yang F, Yan J, van der Veen S. Antibiotic Resistance and Treatment Options for Multidrug-Resistant Gonorrhea. Infectious Microbes and Diseases. 2020;2(2):67-76. doi:10.1097/IM9.0000000000000024
7. Shaskolskiy B, Kandinov I, Dementieva E, Gryadunov D. Antibiotic Resistance in *Neisseria gonorrhoeae*: Challenges in Research and Treatment. Microorganisms. 2022;10(9):1699. doi:10.3390/microorganisms10091699
8. Kandinov I, Dementieva E, Filippova M, Vinokurova A, Gorshkova S, Kubanov A, *et al*. Emergence of Azithromycin-Resistant *Neisseria gonorrhoeae* Isolates Belonging to the NG-MAST Genogroup 12302 in Russia. Microorganisms. 2023;11(5):1226. doi:10.3390/microorganisms11051226
9. Allen VG, Mitterni L, Seah C, Rebbapragada A, Martin IE, Lee C, *et al*. *Neisseria gonorrhoeae* treatment failure and susceptibility to cefixime in Toronto, Canada. JAMA. 2013;309(2):163-170. doi:10.1001/jama.2012.176575
10. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309. doi:10.1179/2047773215Y.0000000030
11. Ikuta KS, Swetschinski LR, Robles Aguilar G, Sharara F, Mestrovic T, Gray AP, *et al*. Global mortality associated with 33 bacterial pathogens in 2019: A systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2022;400:2221-2248. doi:10.1016/S0140-6736(22)02185-7
12. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, *et al*. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet. 2022;399:629-655. doi:10.1016/S0140-6736(21)02724-0
13. Dadgostar P. Antimicrobial resistance: Implications and costs. Infection and Drug Resistance. 2019;12:3903-3910. doi:10.2147/IDR.S234610
14. de Kraker MEA, Stewardson AJ, Harbarth S. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Med. 2016;13:e1002184. doi:10.1371/journal.pmed.1002184
15. Walsh TR, Gales AC, Laxminarayan R, Dodd PC. Antimicrobial Resistance: Addressing a Global Threat to Humanity. PLoS Med. 2023;20(1):e1004264. doi:10.1371/journal.pmed.1004264
16. Jonas OB, Irwin A, Berthe FCJ, Le Gall FG, Marquez P. Drug-resistant infections: A Threat to Our Economic Future. World Bank Rep. 2017;2:1-132. Available: https://www.worldbank.org/en/topic/health/publication/drug-resistant-infections-a-threat-to-our-economic-future
17. World Health Organization. Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. World Heal Organ; c2012. p. 1-40. Available:

https://www.who.int/publications/i/item/9789241503501
18. World Health Organization. Guidelines for the management of symptomatic sexually transmitted infections. World Health Organization; c2021. Available: https://pubmed.ncbi.nlm.nih.gov/34370424/
19. Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, *et al*. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Reports. 2021;70:1-187. doi:10.15585/mmwr.rr7004a1
20. Weston EJ, Workowski K, Torrone E, Weinstock H, Stenger MR. Adherence to CDC Recommendations for the Treatment of Uncomplicated Gonorrhea-STD Surveillance Network, United States, 2016. MMWR Morb Mortal Wkly Rep. 2018;67:473-476. doi:10.15585/mmwr.mm6716a4
21. Romulo A, Zuhud EAM, Rondevaldova J, Kokoska L. Screening of *In vitro* antimicrobial activity of plants used in traditional Indonesian medicine. Pharm Biol. 2018;56:287-293. doi:10.1080/13880209.2018.1462834
22. Mudenda S, Hikaambo CN, Chabalenge B, Mfune RL, Mufwambi W, Ngazimbi M, *et al*. Antibacterial activities of honey against *Escherichia coli* and *Staphylococcus aureus*: A potential treatment for bacterial infections and alternative to antibiotics. J Pharmacogn Phytochem. 2023;12:06-13. doi:10.22271/PHYTO.2023.V12.I3A.14655
23. Hikaambo CN, Chilala P, Ndubi F, Mayoka G, Kampamba M, Kabuka R, *et al*. Antimicrobial Activities of *Solanum aculeastrum* Fruit Extract against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*: Significance of African Traditional Medicine in Combating Infections and Attaining Universal Health Coverage. Pharmacol Pharm. 2023;14:176-188. doi:10.4236/PP.2023.145013
24. Kabuka R, Mudenda S, Kampamba M, Chulu M, Chimombe T, Hikaambo CN. Phytochemical Analysis of Leaf, Stem Bark, and Root Extracts of *Cassia abbreviata* Grown in Zambia. Pharmacol & Pharm. 2022;13:119-128. doi:10.4236/pp.2022.135009
25. Sigidi M, Traore AN, Tshisikhawe MP, Potgieter N. Ethnobotanical evaluation of selected medicinal plants used in treatment of diseases around Venda region: A literature review. Indian J Tradit Knowl. 2017;16:370-377.
26. Mongalo M, Mafoko B. *Cassia abbreviata* Oliv. A review of its ethnomedicinal uses, toxicology, phytochemistry, possible propagation techniques and Pharmacology. African J Pharm Pharmacol. 2013;7:2901-2906. doi:10.5897/ajpp12.1017
27. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A. Agroforestree Database: A tree reference and selection guide version 4.0. Agroforestree Database. 2009;4:20177200441.
28. Dembetembe TT, Lall N, Kritzinger Q. The use of South African medicinal plants in the pursuit to treat gonorrhoea and other sexually transmitted diseases. Medicinal Plants as Anti-infectives: Current Knowledge and New Perspectives. Academic Press; 2022. pp. 183-207. doi:10.1016/B978-0-323-90999-0.00001-X
29. Msiska T, Mwakikunga A, Tembo D, Musopole A, Lampiao F. The *in vivo* Effects of *Cassia abbreviata* Oliv. on Sperm Parameters and Testicular Histology of The Male Wistar Rat. Curr Tradit Med. 2020;7:447-456. doi:10.2174/2215083806999200711162520
30. Kamga-Simo FDY, Kamatou GP, Ssemakalu C, Shai LJ. *Cassia abbreviata* Enhances Glucose Uptake and Glucose Transporter 4 Translocation in C2C12 Mouse Skeletal Muscle Cells. J Evidence-Based Integr Med. 2021;26. doi:10.1177/2515690X211006333
31. Esmear T, Twilley D, Hlati S, Lall N. Flash Talk 2 “Antiproliferative activity of *Elephantorrhiza burkei* Benth. and *Cassia abbreviata* Oliv. traditionally used in South Africa, for lung cancer treatment.” Planta Medica. Georg Thieme Verlag KG; 2022:1396. doi:10.1055/s-0042-1758896
32. Hikaambo CN, Chisanga T, Kamapamba M, Akapelwa TM, Chimombe T, Chulu M, *et al*. Antibacterial Activity of *Cassia abbreviata* Oliv Bark Extract against *Escherichia coli* and *Staphylococcus aureus*. J Pharm Res Sci Technol. 2022;6:76-83. doi:10.31531/jprst.1000161
33. Chitoti JL, Prashar L, Kaonga P. Anti-Diarrhoeal Properties of Stem Bark of *Cassia abbreviata* against Castor Oil-Induced Diarrhoea in Swiss Albino Mice. Med J Zambia. 2021;47:252-260. doi:10.55320/mjz.47.4.732
34. Mulubwa M, Prakash S. Antimicrobial activity and potency of *Cassia abbreviata* oliv stem bark extracts. In: International Journal of Pharmacy and Pharmaceutical Sciences [Internet]. 2015 [cited 22 Jun 2023];9(8):426-428. Available from: https://journals.innovareacademics.in/index.php/ijpps/article/view/5749/7284
35. Klos M, van de Venter M, Milne PJ, Traore HN, Meyer D, Oosthuizen V. *In vitro* anti-HIV activity of five selected South African medicinal plant extracts. J Ethnopharmacol. 2009;124:182-188. doi:10.1016/j.jep.2009.04.043
36. Goire N, Lahra MM, Chen M, Donovan B, Fairley CK, Guy R, *et al*. Molecular approaches to enhance surveillance of gonococcal antimicrobial resistance. Nature Reviews Microbiology. Nat Rev Microbiol; 2014:223-229. doi:10.1038/nrmicro3217
37. Augustino S, Hall JB, Makonda FBS, Ishengoma RC. Medicinal resources of the Miombo woodlands of Urumwa, Tanzania: Plants and its uses. J Med Plant Res. 2011;5:6352-6372. doi:10.5897/JMPR10.517
38. Eloff JN. Quantification the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine. Phytomedicine; 2004:370-371. doi:10.1078/0944711041495218
39. Ritchie MR. Use of herbal supplements and nutritional supplements in the UK: What do we know about their pattern of usage? Proceedings of the Nutrition Society. Proc Nutr Soc; 2007:479-482. doi:10.1017/S0029665107005794
40. Oladunjoye I, Tajudeen Y, Oladipo H, El-Sherbini M. Planetary Health and Traditional Medicine: A Potential Synergistic Approach to Tackle Antimicrobial Resistance. Challenges. 2022;13:24. doi:10.3390/challe13010024