Journal of Pharmaceutical Research Science & Technology

journal homepage: https://ijpp.edwiserinternational.com

Research paper

Antibacterial Activity of Cassia abbreviata Oliv Bark Extract against Escherichia coli and Staphylococcus aureus

Christabel Nang'andu Hikaambo[1,], Tumbila Chisanga[1], Martin Kampamba[1], Tumelo Muyenga Akapelwa[2], Tadious Chimombe[1], Martha Chulu[3], Namuchindo Nanyangwe[4], Reagan Kabuka[3] and Steward Mudenda[1]*

[1]Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, Zambia
[2]Department of Physiological Sciences, School of Medicine and Health Sciences, Mulungushi University, Livingstone, Zambia.
[3]Department of Pharmacy, School of Health Sciences, Levy Mwanawasa Medical University, Lusaka, Zambia
[4]Department of Medicines Control, Zambia Medicines Regulatory Authority, Lusaka, Zambia

ARTICLE INFO

Received 15 January 2022
Revised 28 February 2022
Available Online 05 March 2022

ACADEMIC EDITOR
Dr. Bijay Kumar Mohanty

*CORRESPONDING AUTHOR
Christabel Nang'andu Hikaambo, Department of Pharmacy, School of Health Sciences, University of Zambia, Lusaka, Zambia

ABSTRACT

***Background**: Cassia abbreviata Oliv is believed to possess many pharmacological activities and has been used traditionally to treat many ailments. In Zambia, it is used by traditional healers and the locals to treat various bacterial infections especially in rural areas where traditional medicine is the first or only line of treatment. However, it's phytochemical content and activity on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) has not been documented in Zambia.*

***Objective**: To investigate the phytochemical composition and antibacterial activity of Cassia abbreviata Oliv stem bark extract against Escherichia coli and Staphylococcus aureus.*

***Materials and Methods**: Ethanol and aqueous crude extracts were derived from Cassia abbreviata Oliv stem bark and subjected to qualitative phytochemical screening using standard procedures. The extracts were then used to test for antibacterial activity against standard cultures of E. coli ATCC 25922 and S. ATCC 25923. Ciprofloxacin (5µg) was used as a positive control. The agar disc diffusion was used to determine the antibacterial activity of C. abbreviata at different concentrations (20, 15, 10, 5 and 1mg/mL). The minimum inhibitory concentration (MIC) and zones of inhibition were measured against the tested microorganisms.*

***Results**: The phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, sterols, and phenols. The ethanolic extract was found to have antibacterial activity against S. aureus but not E. coli, while the aqueous extract had no effect on either pathogen. A minimum inhibitory concentration of 5mg/mL was observed with the ethanolic extract. Ciprofloxacin showed better antibacterial activity against both S. aureus and E. coli compared to the extracts.*

***Conclusion**: The ethanolic extract showed a concentration-dependent antibacterial activity against S. aureus while the aqueous extract showed no antibacterial activity.*

***Keywords:** Cassia abbreviata; Phytochemical; Antibacterial; Staphylococcus aureus; Escherichia coli*

Introduction

Herbal antimicrobials have contributed to the control of various diseases caused by microorganisms [1-4]. The World Health Organization (WHO) encourages, recommends, and promotes the inclusion of herbal medicines in national health care systems [5]. This is due to the fact that they are easily available at low rates and are thought to be safer than modern synthetic medications [6].

It has been estimated that 80% of the world's population uses traditional plant medicines to meet their primary health care needs [7]. In Africa, herbal medicines have been found to be effective against various microorganisms that cause infections [8]. In Zambia, traditional medicine and traditional medicinal practices are used by about 70% of the population for primary health care [9-11].

One such plant of vast medicinal value is *Cassia abbreviata*. Cassia is a genus of the family *Caesalpiniacea* and comprises about 600 species [12]. It is well known for its diverse biological and pharmacological properties [13]. *C. abbreviata* is a shrub that grows up to 10 m in height, has a light brown bark, a rounded crown, and yellowish leaves [14]. It is widespread in Africa and occurs mostly at low to medium altitudes (between 220 and 1520 m above sea level), in open bushed, woodland or wooded grasslands, along rivers, on hillsides, and frequently on termite mounds [15].

C. abbreviata has been used in many parts of Africa as a traditional medicine for the treatment of various illnesses, including diabetes mellitus, constipation, headache, diarrhea, skin diseases, fever, infertility, cough, bilharzia, syphilis, malaria and jaundice [16,17]. In Tanzania, a decoction of the root is used for abdominal pains, dysentery, fever, malaria, hernia, wounds, syphilis, impotency, and snake bites [18]. In Mozambique, a decoction of root bark may be taken orally to treat diarrhea and malaria [19].

According to Van Wyk and Gericke, the root of *C. abbreviata* is also used as an aphrodisiac and as an abortifacient [20]. In Zimbabwe, *C. abbreviata* is commonly known as "Muremberembe", and the roots may be crushed, mixed with hot water, and the extract may be drunk to treat disorders like constipation, diarrhoea, venereal diseases, and as an aphrodisiac [21]. A decoction of the stem bark is taken orally to treat stomach aches and malaria, while an infusion of roots, leaves, and stem bark mixed together is taken orally to treat stomachaches [19]. The bark and roots can also be used to treat a mother's stomach aches during pregnancy, newborn babies' close fontanelle, dysentery, blood vomits, venereal diseases, bilharzia, hernia, snake bites, post-partum pains, and menstrual cycle problems [22]. Furthermore, the bark and roots may be used as general blood cleansers and in the treatment of abdominal pains [23]. From the foregoing, *C. abbreviata* could be seen to have antibacterial properties.

C. abbreviata Oliv is also an indigenous plant found in Zambia's forest and is locally called Mululwe by the Ila tribe [24] and has been traditionally used in Zambia in the treatment of leprosy, syphilis, and toothache through the use of an aqueous decoction made from roots or stem barks. The leaves are also smoked as a remedy for haematuria [25].

This study was conducted to investigate the phytochemical composition and antibacterial activity of *C. abbreviata Oliv* stem bark extract against *Escherichia coli* and *Staphylococcus aureus*.

Materials And Methods

Collection of plant material

The stem bark of *C. abbreviata Oliv* was collected from fresh live trees in Chilanga, Zambia in April 2021. The plant was botanically identified and authenticated at the University of Zambia, Department of Biological Sciences.

Preparation of plant extract

The *C. abbreviata Oliv* stem barks were washed, then chopped into small pieces and dried for about 7 days in the shade. The dried barks were pounded into a coarse powder using a motor and pestle. The bark powder was collected and stored in an amber container before being employed in the extraction process.

The solvents used for maceration were 95% ethanol and distilled water. To achieve thorough extraction, 50 g of dried bark powder was placed in two clean empty containers, and 400 ml of solvent (ethanol or water) was added, stirred, closed, and stored for roughly 3 days with occasional stirring. The sample was filtered using Whatman No. 1 filter paper, and the filtrate was evaporated in a water bath at 60°C until a dry extract was obtained. The dry crude extracts were weighed, sealed in an airtight container, and kept at 4°C in the refrigerator.

The percentage yield was calculated as shown in the formula below:

The percentage (%) yield = (weight of final extract) / (weight of stem bark powder) × 100

Phytochemical analysis

The qualitative analysis of *C. abbreviata* stem bark extracts was carried out using standard techniques [11,26]. Simple chemical assays were used to determine the presence of secondary plant elements such as alkaloids, tannins, flavonoids, saponins, terpenoids, sterols, and phenols in the basic phytochemical screening.

Test for Alkaloids

5 mL of 1% aqueous HCl was mixed and agitated with 0.5 g of the ethanolic and aqueous extract on a water bath, then filtered. 2 drops of Wagner's reagent were added to 1mL of filtrate in a test tube and shaken. The presence of alkaloids was indicated by the formation of a reddish-brown precipitate.

Test for Saponins

5 mL distilled water was used to dissolve 0.5 g of the ethanolic and aqueous extract, which was rapidly agitated for 5 minutes. The presence of saponins was determined by persistent frothing that lasted after heat.

Test for Tannins

0.25 g of the ethanolic and aqueous extract was mixed with 5 mL of distilled water before being filtered. 2 drops of 1% ferric chloride solution were added to 5 mL of the filtrate, and the presence of a blue-black, green, or blue-green precipitate confirmed the presence of tannins.

Test for Phenolics

2 mL of 5 % aqueous ferric chloride was added to 2 mL of crude extracts, and the production of violet color indicated the presence of phenols in the sample extract.

Test for Flavonoids

A test tube was filled with 2 mL of each of the ethanolic and aqueous extracts. Then 2 drops of a 10% ferric chloride solution were added. The presence of a phenolic hydroxyl group was identified by a green-blue or violet coloration.

Test for Steroids

2 mL acetic acid was added to 0.2 g of the ethanolic and aqueous extracts; the solution was then chilled thoroughly in ice before carefully adding conc. sulphuric acid. The presence of steroidal ring was indicated by a change in color from violet to blue or bluish green.

Test for terpenoids

In 5 mL ethanol, 0.5 g of extract was dissolved. 1 mL acetic anhydride was added to it, followed by 1 mL conc. sulphuric acid. The presence of terpenoids was indicated by a change in color from pink to violet.

Culturing and collection of the bacteria

Standard bacteria *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were cultured in the microbiology laboratory at the University Teaching Hospital (UTH) in Lusaka, Zambia. Resuscitating the organism in buffered peptone broth and then agar medium and incubating at 37°C for 24 hours brought the cultures to laboratory conditions. Until they were used in the experiment, the bacteria were stored in circumstances that ensured their development and proliferation.

Preparation of bacteria inoculum

4-5 colonies were collected with a wire loop from a pure 24-hour old bacterial isolate culture and transferred to 5mL 0.9% saline (normal saline). The test suspension's turbidity was then virtually compared to 0.5 McFarland turbidity criteria. Under acceptable lighting, the comparison was made against a white background with a contrasting black line. The test suspension's turbidity was adjusted with sterile saline until it reached the 0.5 McFarland standard; equal distortion of the black line indicated turbidity match [27]..

Inoculation

The medium used was Muller-Hinton agar (MHA), and the plates were dried in an incubator at 30-37°C to ensure that any excess moisture was removed before use. A sterile cotton swab was dipped into the standardized bacterial suspension (inoculum), and then pressed against the test tube wall to remove excess inoculum. The agar was then inoculated by streaking it from the middle outwards with the swab holding the inoculum, then rotating it 90 degrees and repeating the rubbing procedure for even distribution of inoculum. Before applying plant extracts, the surface of the medium was allowed to dry for 3-5 minutes to allow excess moisture to be absorbed [27].

Antimicrobial Activity and Minimum Inhibitory Concentration (MIC) Determination

The antibacterial activity and MIC of crude plant extracts against *S. aureus* and *E. coli* bacteria were determined using the disc diffusion method. The positive control was the standard medication Ciprofloxacin (5 g standard disc), while the negative control was 0.5 percent dimethylsulphoxide. The agar plates were stored under sterile conditions for 24 hours after inoculation. To prepare a stock solution, the dried plant extracts were reconstituted in dimethyl sulphoxide to a concentration of 20 mg/mL. After that, the stock solution was diluted to four different concentrations (15, 10, 5, and 1 mg/mL). 50 µl (0.005 mL) of the plant extract was measured using a micropipette, and sterile filter paper discs measuring 5 mm in diameter were soaked in the measured extract. After that, the agar plates were separated into sections and labeled. After that, the agar plates were sectioned and labeled. The discs were spaced apart on the seeded agar plates by at least 24 mm from center to center. For maximum contact with the agar, each disc was pressed down and evenly spread out. The agar discs were then incubated at 37 ° C. for around 24 hours before being examined and the diameter of the zones of inhibition measured in mm with a ruler. Both the aqueous and ethanol extracts were tested against *S. aureus* and *E. coli* during the procedure. The MIC was determined to be the least concentration among the five that inhibited bacterial growth in a zone.

Data analysis

Collected data was analysed using Microsoft Excel 2019. The phytochemical content and antibacterial activity of *C. abbreviata* at different concentrations were recorded, captured and presented in tables. The mean and standard deviations of the zones of inhibition were also obtained using Microsoft Excel 2019.

Ethical consideration

Ethical approval to conduct the study was obtained from the University of Zambia, School of Health Sciences Research and Ethics Committee (UNZAHSREC) through the Department of Pharmacy. The protocol ID was 202112030026. All procedures were done according to the stipulated laboratory guidelines.

Results

Extraction yields

Following the ethanolic and aqueous extractions of the stem bark, the percentage extraction value of *C. abbreviata* extracts were 5.48% and 10.96% respectively. See Table 1.

Table 1: Extraction yields obtained and calculated percentage yield.

Solvent type	Weight of sample before extraction (g)	weight of crude extract (g)	Percentage yield (%)
Ethanol	50	2.74	5.48
Water	50	5.48	10.96

Phytochemical analysis of *C. abbreviata*

Alkaloids, saponins, tannins, phenols, flavonoids, terpenoids, and steroids were found in aqueous extracts of *C. abbreviata* stem bark, while only tannins, phenols, flavonoids, terpenoids, and steroids were found in the ethanolic extract.

Table 2: Phytochemical analysis of aqueous and ethanolic stem bark extracts of *C. abbreviate.*

Phytochemicals	Results	
	Aqueous extract	Ethanol extract
Alkaloids	+	-
Saponins	+	-
Tannins	+	+
Phenols	+	+
Flavonoids	+	+
Terpenoids	+	+
Sterols	+	+

Key: presence (+); absence (-)

Antibacterial activity of aqueous and ethanolic extracts of *C. abbreviata* against *S. aureus* and *E. coli*

The ethanolic extract of *C. abbreviata* stem bark showed antibacterial activity against *S. aureus* with a minimum inhibitory concentration of 5 mg/mL however, no activity against *E. coli was observed*. The aqueous extract did not show any antibacterial activity. Ciprofloxacin had the highest antibacterial activity as shown by the zones of inhibition. See Tables 3 and 4.

Table 3: Mean zone of inhibition of the *C. abbreviata* ethanolic extract.

Concentration of extract, mg/mL		1	5	10	15	20	ciprofloxacin
Bacteria	*E. coli*	0	0	0	0	0	34.7 ± 0.58
	S. aureus	0	6.7+/- 0.58	10.7+/- 0.58	11.7+/- 0.58	13.3+/- 0.58	26.2 ± 0.29

Table 4: Mean Zone of inhibition of the *C. abbreviata* aqueous extract.

Concentration of extract, mg/ml		**1**	**5**	**10**	**15**	**20**	**Ciprofloxacin**
Bacteria	*E. coli*	0	0	0	0	0	34.7 ± 0.58
	S. aureus	0	0	0	0	0	26.2 ± 0.29

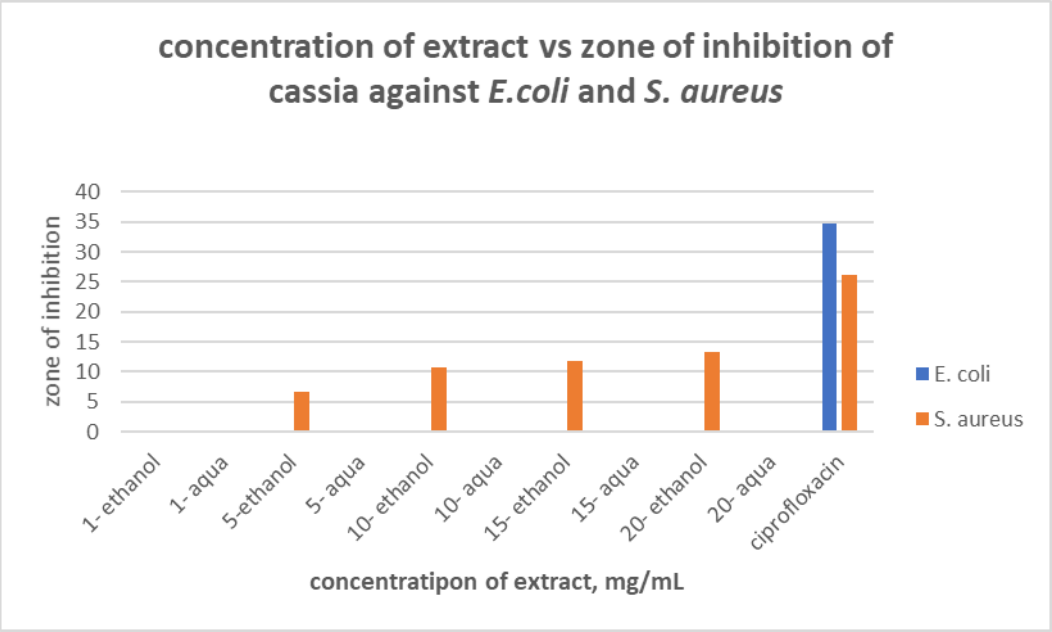

Figure 1: Concentration of extracts versus zone of inhibition of *C. abbreviata* against *E. coli* and *S. aureus*.

Discussion

This study was conducted to investigate the phytochemical composition and antibacterial activity of *cassia abbreviata* stem bark against *E. coli* and *S. aureus*. The phytochemical screening showed that the extracts contained different classes of compounds. The aqueous extract contained all the screened metabolites, which included alkaloids, tannins, flavonoids, saponins, terpenoids, steroids, and phenols, while the ethanolic extract contained the same except alkaloids and saponins. The extracts exhibited several secondary metabolites which may contribute to their antibacterial activity. In a study conducted in Kenya, it was found that the aqueous root bark extract of *C. abbreviata* contained flavonoids, saponins, phenolics, and tannins. However, alkaloids were absent [28]. The variation could be related to the plant's exposure to different geographical and environmental conditions [11]. In a

study reported in Zambia, although a phytochemical analysis was not done, the calculated extractive values showed that water was the solvent that yielded the most quantity of extract [29]. A study by Madureira *et al.* showed varying compositions of phytochemicals depending on the organic solvent used for extraction. In the said study, the phytochemicals under study were alkaloids, flavonoids, phenols, and terpenoids [30]. Differences in the polarity of the solvents can explain the observed variation in phytochemical presence [11].

In this study, the highest zones of inhibition were shown by the ciprofloxacin standard in both bacteria, 26.2 mm for *S. aureus* and 34.7 mm for *E. coli*. The ethanolic extract showed antibacterial activity against *S. aureus* but showed no activity against *E. coli*. This suggests an increased activity of the extract against gram-positive bacteria compared to gram-negative bacteria. Similarly, a study conducted in Tanzania on the ethanolic extract of *C. abbreviata* revealed that it had minimal antibacterial activity against both *S. aureus* and *E. coli* [12]. However, a study conducted on a similar plant species, *Cassia fistula,* growing in Zambia, indicated activity against *E. coli, P. aeruginosa,* and non-typhoidal *salmonella* [29], while a study in India indicated that the plant *Cassia fistula* had activity against both gram positive and gram negative bacteria, although there was superior activity against the gram negative bacteria [31]. Further, in a study done in Zimbabwe, ethanolic pod extract showed antibacterial activity against *E. coli* [32]. The observed differences could be as a result of the use of different plant parts, e.g., the use of pods while this study used leaves, as well as different sources of the plant collected. Most plant extracts are believed to be more active against gram-positive bacteria because the cell wall is easier to penetrate than gram-negative ones, which contain an outer membrane with a lipopolysaccharide layer that is impermeable to certain antibiotics and antibacterial compounds [33].

The ethanolic extract had better activity than the aqueous extract, which showed no antibacterial activity in both *S. aureus* and *E. coli*. This suggests that ethanol is a better extraction solvent than water due to the lower extraction of antimicrobial compounds into the aqueous extract. The current findings also suggest that ethanolic extracts are rich in flavonoids which are responsible for antimicrobial activities [34].

Similarly, Seyyednejad *et al*., reported that the ethanolic extract had superior activity in comparison to the methanolic extract [31]. Further, in the study reported by Muyenga *et al*., the aqueous extract did not show any superiority over the methanolic extract [29]. However, there was no statistically significant difference in the antibacterial activity of either solvent used. It was reported that this was due to the lower extraction of antimicrobial compounds into the aqueous extract or to minimum availability of the aqueous extract to the microorganism.

These study findings revealed that *C. abbreviata's* antibacterial activity is due to the presence of secondary metabolites. Plant phytochemical compounds such as alkaloids, saponins, tannins, flavonoids, and steroids, which have been known to be biologically active and thus partially responsible for the antimicrobial activities of plants, hence their use in traditional medicine [35]. Herbal antibiotics must be used prudently for they may be the solution to tackle antibiotic-resistant microorganisms [8,36-40].

Conclusion

The ethanolic extract of *Cassia abbreviata* stem bark extract was found to have antibacterial activity against *S. aureus* but not against *E. coli*. The zone of inhibition increased as the concentration of the extract increased, demonstrating that the extract's antibacterial activity was proportional to its concentration. The aqueous extract, on the other hand, had no effect on the bacteria.

Consent for Publication

Not applicable.

Funding

The authors did not receive any financial sponsorship for the research.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgements

None declared.

References

1. Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites. 2019;9(11):258.
2. Chassagne F, Samarakoon T, Porras G, Lyles JT, Dettweiler M, Marquez L, et al. A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective. Frontiers in pharmacology. 2021:2069.

3. Mintah SO, Asafo-Agyei T, Archer M-A, Junior PA-A, Boamah D, Kumadoh D, et al. Medicinal plants for treatment of prevalent diseases. Pharmacognosy-Medicinal Plants. 2019.
4. Karen W. Martin EE. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. Journal of Antimicrobial Chemotherapy. 2003;51(2):241–6.
5. WHO. WHO global report on traditional and complementary medicine 2019: World Health Organization; 2019.
6. Alam G, Mishra AK. Traditional and Modern Approaches for Standardization of Herbal Drugs: A Review. Acta Biomedica Scientia e-ISSN. 2017;2348:2168.
7. WHO. WHO traditional medicine strategy 2002–2005. Geneva: World Health Organization; 2002. 2017.
8. Walusansa A, Asiimwe S, Nakavuma J, Ssenku J, Katuura E, Kafeero H, et al. Antibiotic-resistance in medically important bacteria isolated from commercial herbal medicines in Africa from 2000 to 2021: a systematic review and meta-analysis. Antimicrobial Resistance & Infection Control. 2022;11(1):1-20.
9. Hikaambo CN, Namutambo Y, Kampamba M, Mufwambi W, Kabuka R, Chulu M, Nanyangwe N, Banda M, Chimombe T, Muungo LT, Mudenda S. Prevalence and Patterns of Herbal Medicine Use among Type 2 Diabetes Mellitus Patients at the University Teaching Hospitals in Lusaka. J Biomed Res Environ Sci. 2022 Jan 20; 3(1): 074-081.
10. Muyenga T, Musonda D, Chigunta M. Ethnobotanical survey of medical plants used in treatment of diabetes in Chipulukusu compound, Ndola district, Zambia. J Prev Rehabil Med. 2018;1:39-44.
11. Hikaambo CN, Kaacha L, Mudenda S, Nyambe MN, Chabalenge B, Phiri M, et al. Phytochemical Analysis and Antibacterial Activity of Azadirachta indica Leaf Extracts against Escherichia coli. Pharmacology & Pharmacy. 2022;13(1):1-10.
12. Kirabo I, Mabiki FP, Mdegela RH, Obbo CJ. In vitro antibacterial potential of extracts of Sterculia africana, Acacia sieberiana, and Cassia abbreviata ssp. abbreviata used by yellow baboons (Papio cynocephalus) for possible self-medication in Mikumi National Park, Tanzania. International Journal of Zoology. 2018;2018.
13. Chanda S, Kaneria M, Baravalia Y. Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four Cassia species. African Journal of Biotechnology. 2012;11(10):2490-503.
14. Mongalo N, Mafoko B. Cassia abbreviata Oliv. A review of its ethnomedicinal uses, toxicology, phytochemistry, possible propagation techniques and Pharmacology. African Journal of Pharmacy and Pharmacology. 2013;7(45):2901-6.
15. Pawar HA, D'mello PM. Cassia tora Linn.: an overview. International journal of pharmaceutical sciences and research. 2011;2(9):2286.
16. Bati K, Kwape TE, Chaturvedi P. Anti-Diabetic effects of an ethanol extract of cassia abbreviata stem bark on diabetic rats and possible mechanism of its action:-Anti-diabetic properties of cassia abbreviata. Journal of Pharmacopuncture. 2017;20(1):45.
17. Kamga-Simo III F, Kamatou G, Ssemakalu C, Shai L. Cassia Abbreviata Enhances Glucose Uptake and Glucose Transporter 4 Translocation in C2C12 Mouse Skeletal Muscle Cells. Journal of evidence-based integrative medicine. 2021;26:2515690X211006333.
18. Muthaura C, Rukunga G, Chhabra S, Mungai G, Njagi E. Traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast. Journal of ethnopharmacology. 2007;114(3):377-86.
19. Ribeiro A, Romeiras MM, Tavares J, Faria MT. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. Journal of ethnobiology and ethnomedicine. 2010;6(1):1-15.
20. Van Wyk B-E, Gericke N. People's plants: A guide to useful plants of Southern Africa: Briza publications; 2000.
21. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. Journal of ethnobiology and ethnomedicine. 2013;9(1):1-18.
22. Bruschi P, Morganti M, Mancini M, Signorini MA. Traditional healers and laypeople: a qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). Journal of Ethnopharmacology. 2011;138(2):543-63.
23. Mojeremane W, Legwaila G, Mogotsi K, Tshwenyane S. Monepenepe (Cassia abbriviata): A Medicinal Plant in Botswana [2005]. 2005.
24. Fowler DG. Traditional ila plant remedies from Zambia. Kirkia. 2002:35-48.
25. Mulubwa M, Prakash S. Antimicrobial activity and potency of cassia abbreviata Oliv stem bark extracts. Int J Pharm Pharm Sci. 2015;7:426-8.

26. Evans WC. Trease and evans. Pharmacognosy, 9th Edition published by Saunders Elsevier. 2002:553-7.
27. Masaiti GC, Malambo C, Hikaambo C, Banda M, Matafwali SK, Mufwambi W, et al. Antibacterial properties of ficus sycomorus bark extract against Staphylococcus aureus and Escherichia coli. International Journal of Biomedical Investigation. 2019;2(2):1-7.
28. Njagi S, Lagat R, Mawia A, Arika W, Wambua F, Ouko R, et al. In vitro antiproliferative activity of aqueous root bark extract of cassia abbreviata (holmes) brenan. J Cancer Sci Ther. 2016;8:114-21.
29. Muyenga T, Hamweene P, Chabala F, Hamooya B. Antimicrobial activity of Cassia fistula Linn. stem bark extracts on Escherichia coli, Pseudomonas aeruginosa and Nontyphoidal Salmonella. Pharmaceutical Journal of Zambia 2021;5(1).
30. Madureira AM, Ramalhete C, Mulhovo S, Duarte A, Ferreira M-JU. Antibacterial activity of some African medicinal plants used traditionally against infectious diseases. Pharmaceutical Biology. 2012;50(4):481-9.
31. Seyyednejad SM, Motamedi H, Vafei M, Bakhtiari A. The antibacterial activity of Cassia fistula organic extracts. Jundishapur Journal of Microbiology. 2014;7(1).
32. Mujuru S. Flavonoid content, anti-bacterial and anti-inflammatory activity of cassia abbreviata pods: BUSE; 2018.
33. Wintola OA, Afolayan AJ. The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of Hydnora africanaThunb. used as antidysenteric in Eastern Cape Province, South Africa. BMC complementary and alternative medicine. 2015;15(1):1-12.
34. Ahmed SI, Hayat MQ, Tahir M, Mansoor Q, Ismail M, Keck K, et al. Pharmacologically active flavonoids from the anticancer, antioxidant and antimicrobial extracts of Cassia angustifolia Vahl. BMC complementary and alternative medicine. 2016;16(1):1-9.
35. Nethathe B, Ndip R. Bioactivity of Hydnora africana on selected bacterial pathogens: Preliminary phytochemical screening. African Journal of Microbiology Research. 2011;5(18):2820-6.
36. Gupta P, Birdi T. Development of botanicals to combat antibiotic resistance. J Ayurveda Integr Med 8 (4): 266–275. 2017.
37. Jahangir MA, Khan S, Singh AD, Muheem A, Soni A, Taleuzzaman M. Nanophytomedicine in clinical management: An introductory evidence-based review. Journal of Pharmaceutical Research Science & Technology. 2022 Feb 7;6(1):26-37.
38. Jahangir MA, Khan S, Kala C. Phytonutrients and Technological Development in Formulations. J Pharm Res Sci Technol 2022; 6 (1): 159. doi: 10.31531/jprst.;1000159:39.
39. Jahangir MA, Muheem A, Imam SS, Gilani SJ, Zafar A, Alshehri S, Jafar M. High Altitude Edible Plants: A Great Resource for Human Health and their Socio-Economic Significance. InEdible Plants in Health and Diseases 2022 (pp. 161-180). Springer, Singapore.
40. Jahangir MA, Muheem A, Imam SS, Ahmed FJ, Aqil M. Nigella sativa Encapsulated Nano-Scaffolds and Their Bioactivity Significance. InBiomarkers as Targeted Herbal Drug Discovery. 2021 Jul 4:155-75.