Journal of Medicinal Plants for Economic Development
ISSN: (Online) 2616-4809, (Print) 2519-559X

A review of *Zanthoxylum chalybeum* Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicology

Authors:
Scelo Mguni[1]
Felistas Mashinya[2]
Collette Khabo-Mmekoa[1]
Lishweni J. Shai[1]

Affiliations:
[1]Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa

[2]Institute of Bio Research Auditing and Training in Southern Africa, Pretoria, South Africa

Corresponding author:
Scelo Mguni,
scelomguni@gmail.com

Dates:
Received: 09 Mar. 2023
Accepted: 22 July 2023
Published: 29 Sept. 2023

How to cite this article:
Mguni, S., Mashinya, F., Khabo-Mmekoa, C. & Shai, L.J., 2023, 'A review of *Zanthoxylum chalybeum* Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicology', *Journal of Medicinal Plants for Economic Development* 7(1), a202. https://doi.org/10.4102/jomped.v7i1.202

Background: *Zanthoxylum chalybeum* Engl. is a traditional medicinal plant, which is native to Eastern and Southern Africa. Commonly known as the 'Knob wood', it has been used for centuries by several traditional healers in Kenya, Tanzania, Uganda, Zambia and Zimbabwe. The species is very well known to local communities by its common names such as 'Kundanyoka' (Shona), 'Mjafari' (Swahili) and 'Ntaleyedungu' (Uganda), and it grows naturally in the tropics and subtropics.

Aim: The present review describes information on the ethnomedicinal uses, phytochemical constituents, pharmacology and toxicology of *Z. chalybeum*.

Method: Collection of data was based on literature research from several sources including electronic databases such as Google scholar, Web of Science, Science Direct, Pubmed, books, book chapters and theses.

Results: *Z. chalybeum* is widely used in the treatment of malaria, cancer and diabetes. Pharmacological studies revealed that crude extracts and some isolated chemical compounds from *Z. chalybeum* demonstrated biological activities such as antibacterial, antidiabetic and antimalarial activities. Studies in phytochemical analysis of *Z. chalybeum* revealed the presence of new compounds such as 6-benzo (1, 3) dioxol-5-yl-hexa-2,5 dienoic acid isobutylamide, 4-methoxy-N-(2-methoxy-phenyl)-N methyl-benzamide, N-(2-hydroxy-methyl-propyl)-3 phenyl-acrylamide and 4-(isoprenyloxy)-3-methoxy,4-deoxymethylenedioxyfagaramide. Toxicology studies revealed moderate to high toxicity, depending on the type of cells and the extraction solvent used.

Conclusion: *Z. chalybeum* is a valued medicinal plant used in Eastern and Southern Africa.

Contribution: The properties of *Z. chalybeum* revealed in previous studies can be used to guide research scientists in future drug formulations.

Keywords: *Zanthoxylum chalybeum* Engl; toxicology; pharmacology; ethnobotanical; phytochemical compound.

Introduction

Plant extracts have always been used by people in various parts of Africa and Asia for the treatment of livestock and human ailments (Kokwaro 2009). According to the World Health Organization (WHO) (WHO 2001), the African and Asian communities relied on traditional medicine, especially plant-based remedies to meet their primary healthcare needs. The use of plant-derived medicines is preferred because of their affordability and safety in comparison to synthetic alternatives. Because most conventional drugs are expensive and inaccessible to locals living far away from health centres, this ever-rising cost of modern medicine has prompted most people in developing countries to resort to traditional medicine to treat a variety of ailments. Hence, decoctions, concoctions and infusions from different parts of medicinal plants are being used.

Zanthoxylum chalybeum Engl var *chalybeum* is a deciduous plant of the tropics and subtropics and native to the Eastern and parts of Southern African countries (Ethiopia, Somalia, Kenya, Uganda, DRC, Tanzania, Lesotho, South Africa, Rwanda, Malawi, Burundi, Namibia, Zambia, Mozambique, Eswatini and Zimbabwe) (Figure 1). It is commonly known as the 'Knob wood', because of its characteristic large, conical woody knobs, with sharp thorns along its trunk (Orwa et al. 2009).

Source: Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons, A., 2009, *Agroforestree database: A tree reference and selection guide version 4.0*, World Agroforestry Center, Nairobi

FIGURE 1: Distribution of *Zanthoxylum chalybeum* in native Africa.

Across Africa, *Z. chalybeum* has been used by community elders and traditional healers for the treatment of diseases, and it is prepared and used in a variety of different ways. In Kenya, it is one of the most commonly used traditional plants, and it is used to treat ailments such as rheumatism, sexually transmitted infections, amoebiasis and throat infections (Kipkore et al. 2014; Kiringe 2006). In Tanzania, the plant is used for treating malaria, jaundice, heart infections and pneumonia (Chhabra et al. 1990; Mbinile et al. 2020). In Uganda, Zambia and Zimbabwe, it is used to treat sickle cell anaemia, skin diseases and dental diseases, respectively (Chagonda et al. 1994; Chisowa, Hall & Farman 1999; Engeu et al. 2008). The plant parts mainly used are the stem bark, root bark and leaves. They are either ground to powder, burnt or prepared for decoctions or concoctions that can be drunk, chewed, applied topically or mixed with other liquids such as tea or milk.

Zanthoxylum chalybeum has been previously studied for its pharmacological properties. Research has shown tremendous results on the antibacterial, antifungal, antiplasmodial, antidiabetic, anticancer and antisickling properties of *Z. chalybeum* (Agwaya, Natundu & Vuzi 2016; Engeu et al. 2008; Njenga et al. 2016; Omosa et al. 2021; Pierre, Munyabuhoro & Emmanuel 2011; Taniguchi et al. 1978). Analysis of active chemical compounds of *Z. chalybeum* has shown the presence of reducing sugars, alkaloids, anthracenosides, coumarine derivatives, flavonoids, steroid glycosides, triterpenes, anthocyanocides, saponins and cardiac glycosides (Adia et al. 2016; Nalule, Mbaria & Kimenju 2013).

Studies on the safety levels of *Z. chalybeum* have generally established that the plant has fairly low to moderate toxicity. However, some researchers have reported high toxicity in some plant parts using the brine shrimp lethality assay (Chrian et al. 2011; Nguta et al. 2011). The study aims to review the ethnomedicinal uses, pharmacological properties, phytochemical and toxicology of *Z. chalybeum*. An understanding of *Z. chalybeum* from science-backed evidence may allow for potentially new drugs that can address the growing problem of multi-drug resistant pathogens.

Methods

Relevant information on the ethnobotanical uses, pharmacological, phytochemistry and toxicology of *Z. chalybeum* was collected from various scientific studies. A search using the web search engine Google and other databases of scientific journals such as PubMed (https://pubmed.ncbi.nlm.nih.gov/), Science direct (www.sciencedirect.com), Web of Science (www.webofknowledge.com), Springerlink (www.link.springer.com) and google scholar (www.scholar.google.com) were used to retrieve valuable information. Keywords such as *Z. chalybeum*, ethnomedicinal, phytochemistry, toxicity and pharmacology were used to collect relevant information. Other relevant scientific publications were obtained from the Tshwane University of Technology library, South Africa, in order to include books, theses and scientific write-ups with known academic rating. Studies were not limited to a time frame as this review has never been done. Criteria were set to screen the search results for relevance in the study. Only scientific articles published in English were considered. Exclusion criteria were publications containing no original research, samples collected outside Africa and studies that excluded *Z. chalybeum*.

Review findings

Search criteria

Online search strategy identified 122 articles. Articles not meeting the selection criteria (59) and those that were not written in English (2) were excluded. Thirteen articles were added after manual search from retrieved articles. A total of 74 relevant articles were chosen and full text manuscripts were read. Sixty-three articles met the inclusion criteria, 19 articles yielded information on ethnobotanical uses, 15 on pharmacological uses, 6 on phytochemical, 5 on toxicological and 18 on more than one category. Figure 2 shows a schematic depicting of the study selection process. Table 1 to Table 4 summarise the results on ethnomedicinal, pharmacological, phytochemical and toxicological properties of *Z. chalybeum* studied in this review.

Ethnomedicinal uses of Zanthoxylum chalybeum

A total of 35 ethnomedicinal uses of *Z. chalybeum* are documented in the literature (Table 1), from eight countries. Tanzania had the most ethnomedicinal uses (13), followed by Kenya (12), Uganda (6), Zambia (5), Zimbabwe (3), Somalia

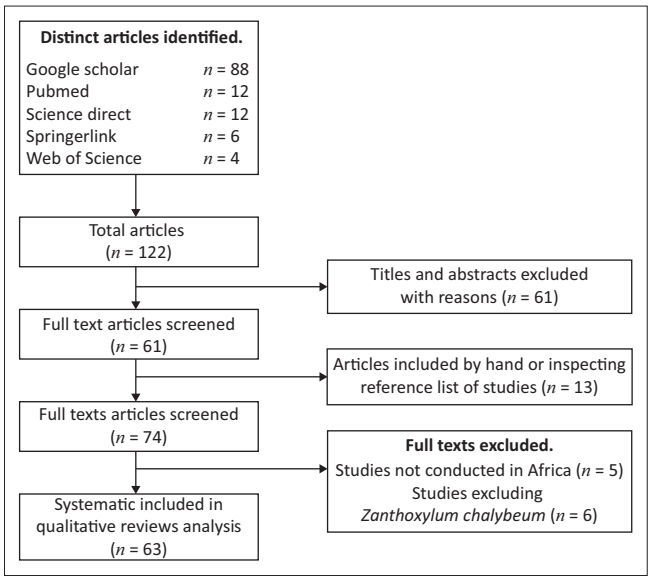

FIGURE 2: Flow diagram of study selection process.

(2), Rwanda (1) and Ethiopia (1). In Uganda, Kenya and Tanzania, *Z. chalybeum* was most frequently used ethnobotanically to treat malaria. The most widely used plant parts were the roots (23), followed by the stem (12), leaves (8) and lastly berries (3) (Table 1). According to literature, *Z. chalybeum* decoctions or concoctions can be boiled and then drank alone or mixed with other beverages like tea or milk (Kiringe 2006). In Kenya, the bark and seeds of *Z. chalybeum* are combined with most traditional preparations as a synergistic herb to improve efficiency (Kiringe 2006). Matata et al. (2018) established that *Z. chalybeum* is used to treat breast and cervical cancer, and the people of Mkuranga and some districts in Tanzania use the stem and root barks to prepare the concoctions. *Zanthoxylum chalybeum* can be mixed with plant parts of other medicinal plants; for example, the bark can be used alone or in combination with *Terminalia spinose* in the treatment of malaria (Mbinile et al. 2020). It can also be mixed with different parts of *Zehneria scabra*, and the concoction is administered after surgery (Kipkore et al. 2014). Table 1 lists the ethnomedicinal applications of *Z. chalybeum* in Africa.

Pharmacological properties of Zanthoxylum chalybeum

Studies have demonstrated that *Z. chalybeum* exhibits antimicrobial activity for some microorganisms (Chrian et al. 2011; Taniguchi et al. 1978). Pierre et al. (2011) revealed that the ethanolic extract showed activity against *Salmonella typhimurium, Pseudomonas aeruginosa* and *Staphylococcus aureus* and reduced the growth of Gram-negative *S. typhi* and *P. aeruginosa*. Maima and Munyendo (2018) established antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), while Schultz et al. (2020) established that *Z. chalybeum* root bark displayed high inhibitory activities against two multidrug-resistant ESKAPE pathogens, *Enterococcus faecium* (MIC 32 µg/mL) and *S. aureus* (MIC 16 µg/mL). Interestingly, Mahamadi and Wunganayi (2018) conducted a study on the

TABLE 1: Ethnomedicinal uses of *Zanthoxylum chalybeum* in Africa.

Plant part	Country	Ethnomedicinal use	Reference
Roots	Uganda	Sickle cell anaemia	Engeu et al. (2008)
Leaves and roots, boiled and drunk	Kenya	Joint conditions	Wambugu et al. (2011)
Parasitic plants and fruiting bodies, fruit berries, higher parasite can be burnt and ashes licked.	Kenya, Zambia, Tanzania	Rheumatism	Kipkore et al. (2014) Chisowa et al. (1999) Iloki-Assanga (2015)
Stem bark mixed with *Zheneria scabra* plant and drunk or simply boiled and drunk.	Kenya	After surgery	Kipkore et al. (2014)
Seed extract	Uganda	Measles	Olila, Olwa-Odyek and Opuda-Asibo (2002)
Root powder mixed with root powder of *Suregada zanzibariensis*	Tanzania	Asthma	Hedberg et al. (1983)
Root bark decoction	Zambia	Diarrhoea	Chisowa et al. (1999)
Leaves, roots – boiled and drunk	Somalia, Tanzania	Abdominal pains	Mbinile et al. (2020); Chhabra et al. (1990)
Leaves	Somalia	Urinary retention	Mbinile et al. (2020)
Leaves and roots. Roots are pounded, water added, then drunk. Leaves are dried, powdered, cold macerated and drunk	Ethiopia, Uganda	Cancer	Tuasha, Petros and Asfaw (2018a); Tuasha, Petros and Asfaw (2018b); Omosa et al. (2021)
Plant juice added to milk	Kenya, Tanzania	Appetite in children	Medhi, Deka and Bhau (2013)
Root bark, leaves	Tanzania	Jaundice	Moshi and Mwambo (2002)
Root bark, leaves, stem bark boiled and drunk	Tanzania	Pain relief	Moshi and Mwambo (2002); Mbinile et al. (2020)
Roots, dried and powdered, then mixed with tea or porridge	Tanzania	Heart infections	Chhabra et al. (1990)
Stem bark or roots, boiled and drunk while hot	Tanzania	Pneumonia	Chhabra et al. (1990); Mbinile et al. (2020);
Stem bark	Kenya, Tanzania	Diabetes	Kimani et al. (2015)
Root, used to brush teeth, stem – chewed	Uganda, Zimbabwe	Dental caries and tooth aches, dental issues	Kokwaro (2009); Chagonda et al. (1994)
Root, applied topically or orally	Tanzania	Fungal infections	Moshi et al. (2007)
Root decoction, stem bark decoction, leaves	Uganda, Kenya, Tanzania	Malaria	Neuwinger (1996); Njoroge and Bussmann (2006); Omara (2020); Kokwaro (1993); Tabuti (2008); Kipkore et al. (2014); Mbinile (2020); Kiraithe et al. (2016)
Root	Tanzania	Whooping cough	Chhabra et al. (1990)
Root	Uganda	Tuberculosis	Tabuti, Kukunda and Waako (2010)
Roots, boiled in water and drunk	Kenya	Sexually transmitted infections	Kiringe (2006)
Roots, outer cover removed, boiled in water or mixed with tea, then sieved and drunk	Kenya	Flu or colds	Kiringe (2006)
Roots, outer cover removed, crushed then boiled in water.	Kenya	Throat infections	Kiringe (2006)
Stem bark, seeds. Boiled or chewed	Kenya	Amoebiasis	Kipkore et al. (2014)
Roots – boiled, filtered and drunk	Kenya, Zambia	Cholera	Jolly, Jefwa and Mwafaida (2017); Chisowa et al. (1999)
Stem, boiled, filtered and drunk	Kenya	Typhoid fever	Jolly, Jefwa and Mwafaida (2017)
Roots and stem bark mixture are boiled and juice is drunk while hot	Tanzania	Women health system	Mbinile et al. (2020)
Roots are boiled and drunk	Tanzania	Dizziness	Mbinile et al. (2020)
Flowers and seeds, dried, ground to powder and mixed with other beverages and drunk daily	Tanzania	Body immunity	Mbinile et al. (2020)
Leaves chewed while raw, stem barks are boiled and juice is drunk	Tanzania	Chest pains	Mbinile et al. (2020)
Roots	Zambia	Gonorrhoea	Chisowa et al. (1999)
Berries	Zambia	Skin diseases	Chisowa et al. (1999)
Root bark	Zimbabwe, Tanzania	Swellings	Chagonda et al. (1994); Chhabra et al. (1990)
Root bark	Zimbabwe, Rwanda	Fever	Chagonda et al. (1994); Pierre et al. (2011)

Note: Please see the full reference list of the article, Mguni, S, Mashinya, F, Khabo-Mmekoa, C. & Shai, L.J., 2023, 'A review of Zanthoxylum chalybeum Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicology!.', *Journal of Medicinal Plants for Economic Development* 7(1), a202. https://doi.org/10.4102/jomped.v7i1.202, for more information.

development of silver nanoparticles using root extracts of *Z. chalybeum*, where *Z. chalybeum* was used both as a reducing and stabilising agent. Their results showed that silver nanoparticles synthesised using *Z. chalybeum* increased the zones of inhibition against *Bacillus subtilis*, *Escherichia coli* and *P. aeruginosa* as compared to the control antibiotic drug and the plant extract alone, and the root extract alone also exhibited a satisfactory result. However, some studies have established that bacteria such as *E. coli* cannot be inhibited by *Z. chalybeum* extracts (Kaigongi et al. 2014; Nguta & Kiraithe 2019). Olila, Olwa-Odyek and Opuda-Asibo (2001) demonstrated that all *Z. chalybeum* stem bark and seed ethanolic, petroleum ether and aqueous extracts did not exhibit antimicrobial activity against *E. coli*, *S. aureus* and *Candida albicans*. Likewise, Nguta and Kiraithe (2019) observed no antibacterial activity against *E. coli*. *Zanthoxylum chalybeum* has also been studied for its antiplasmodial properties. Recently, Mollel et al. (2022) reported antiviral activity of *Z. chalybeum* root bark against the respiratory syncytial virus (RSV), human parainfluenza virus 2 (HPIV-2) and the herpes simplex virus 2 (HSV-2). Njenga et al. (2016) and Waiganjo et al. (2020) revealed significant antiplasmodial activity against drug- sensitive and drug-resistant strains of *P. falciparum*. Studies have also revealed noteworthy antidiabetic (Agwaya & Natundu 2016; Agwaya et al. 2016; Nyongesa 2019), anticancer (Omosa et al. 2021) and anti- sickling (Engeu et al. 2008) activity of *Z. chalybeum*. Table 2 depicts the reviewed pharmacological properties of *Z. chalybeum* in African countries.

TABLE 2: Pharmacological properties of *Zanthoxylum chalybeum*.

Pharmacological activity	Plant part and solvent used	Organisms	Findings	Author
Antibacterial	Stem bark, aqueous extract	MRSA (ATCC 2913)	Antibacterial activity exhibited	Maima and Muyendo (2018)
Antibacterial	Root, aqueous extract	MRSA, *E. coli*, *S. aureus* and *B. cereus*	Antibacterial activity against *B. cereus, S. aureus* and MRSA but not *E. coli*.	Nguta and Kiraithe (2019)
Antibacterial	Root bark, Chloroform: methanol (1:1) and aqueous	*B. cereus, Pseudomonas aeruginosa, E. coli*, MRSA.	Chloroform methanol extract exhibited antibacterial activity against *P. aeruginosa, B. cereus*, MRSA. Aqueous extract activity against *B. cereus*	Kaigongi et al. (2014)
Antibacterial	Stem bark, ethanol, Dichloromethane, Acetone	*E. coli*, *P. aeruginosa*, *S. typhi*, *S. aureus* and *C. albicans*	Ethanolic extract exhibited antibacterial activity against *S. typhi*, *P. aeruginosa* and *S. aureus.* Dichloromethane extract – antibacterial activity against *S. aureus.* Acetone extract – no inhibition	Pierre et al. (2011)
Antibacterial	Stem bark and seed ethanolic, petroleum ether and aqueous extracts	*E. coli*, *S. aureus* and *C. albicans*	No antibacterial activity	Olila, Olwa-Odyek and Opuda-Asibo (2001)
Antibacterial	Silver nanoparticles using root bark, aqueous	*B. subtilis*, *E. coli* and *P. aeruginosa*	Antibacterial activity exhibited	Mahamadi and Wunganayi (2018)
Antibacterial	Root bark, diethyl ether	MDR *E. faecium* and *S. aureus*	High inhibitory activities displayed *E. faecium* MIC 32 µg/mL and *S. aureus* 16 µg/mL	Schultz et al. (2020)
Mycobacterial	Stem bark, Dichloromethane, Methanol	*M. madagascariense* and *M. indicus pranii.*	Antibacterial activity exhibited	Chrian et al. (2011)
Antifungal	Root bark, Methanol	*P. crustosum* and *S. cerevisae,*	Antifungal activity exhibited	Taniguchi et al. (1978)
Antiplasmodial	Stem bark and root bark Dichloromethane: chloroform (1:1)	Chloroquine sensitive (D6) and chloroquine resistant (W2) *P. falciparum*	Stem bark IC_{50} 6 µg/mL Root bark IC_{50} 0.78 µg/mL	Njenga et al. (2016)
Antiplasmodial	Leaf, aqueous, methanol and dichloromethane	Siera – Leonean chloroquine sensitive and Indochinese chloroquine-resistant *strains P falciparum*	Moderate inhibition exhibited by all extracts	Waiganjo et al. (2020)
Antiplasmodial	Leaf, ether and methanol	*P. falciparum* schizonts	Antiplasmodial activity exhibited	Bbosa et al. (2014)
Antiplasmodial	Stem bark, Aqueous and CH_3:MeOH	*P. burghei* in Swiss albino mice	Antiplasmodial activity exhibited	Musila (2012)
Antiplasmodial	Root bark Aqueous, methanol	Chloroquine-sensitive (NF54) and chloroquine-resistant (ENT30) *P. falciparum*	Antiplasmodial activity exhibited, all having IC_{50} < 6 µg/mL	Rukunga et al. (2009)
Antiplasmodial	Root bark, Methanol	*P. falciparum*, chloroquine sensitive (3D7) and chloroquine resistant (W2)	Antiplasmodial activity exhibited	Muganga et al. (2010)
Antiplasmodial	Stem bark fagaramide, ethylacetate and methanol	Chloroquine sensitive (NF54) and chloroquine resistant (FCR3) *P. falciparum*	All active against the chloroquine-resistant strain	Adia et al. (2016)
Antiviral	Seeds, Skimmianine	Schwartz measles strain	Antiviral activity exhibited	Olila, Olwa-Odyek and Opuda-Asibo (2002)
Antiviral	Root bark, Aqueous	Respiratory syncytial virus (RSV)	Moderate antiviral activity exhibited	Mollel et al. (2022)
Antidiabetic	Root bark, Aqueous	Myocardial dysfunction in alloxan-induced type 1 diabetic rats (200 mg/kg and 400 mg/kg)	Blood glucose level significantly reduced for 400 mg/kg, significant increases HDL cholesterol and significant decrease the levels of triglycerides. Therefore, significant reductions in blood glucose levels	Agwaya and Natundu (2016)
Antidiabetic	Root bark, Aqueous	Alloxan-induced diabetic rats	Significant reduction in blood glucose levels. Regeneration and increase in number of the central β cells in diabetic rats	Agwaya et al. (2016)
Antidiabetic	Stem bark, Aqueous	Streptozotocin-induced diabetic rats	Significant antidiabetic effects	Kimani et al. (2015)
Antidiabetic	Root and stem bark, methanol and ethyl acetate extracts	α amylase and α glucosidase enzymes	All extracts exhibited noteworthy inhibition	Nyongesa (2019)
Antidiabetic	*Z. chalybeum* alkaloids from root bark	α amylase and α glucosidase enzymes	Significant inhibition established.	Ochieng et al. (2020)
Anticancer	4-(isoprenyloxy)-3-methoxy-3, 4-dioxymethelenedioxy fagaramide	Drug-sensitive leukaemia CCRF-CEM, multidrug-resistant P-glycoprotein-over-expressing CEM /ADR5000 cells	Moderate inhibition against the two cell lines exhibited	Omosa et al. (2021)
Antisickling	Root bark, ethanol acetic acid 9:1	Male albino Wistar rats	No death and no remarkable changes in general appearance and animal behaviour	Engeu et al. (2008)

Note: Please see the full reference list of the article, Mguni, S, Mashinya, F, Khabo-Mmekoa, C. & Shai, L.J., 2023, 'A review of Zanthoxylum chalybeum Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicologyl.', *Journal of Medicinal Plants for Economic Development* 7(1), a202. https://doi.org/10.4102/jomped.v7i1.202, for more information.

HDL, high-density lipoprotein; MRSA, Methicillin-resistant *Staphylococcus aureus*; MDR, multi drug resistant; MIC, minimum inhibitory concentrations; *E. coli, Escherichia coli; S. aureus, Staphylococcus aureus; B. cereus, Bacillus cereus; P. aeruginosa, Pseudomonas aeruginosa; S. typhi, Salmonella typhimurium; C. albicans, Candida albicans; P. falciparum, Plasmodium falciparum; P. burghei, Plasmodium burghei; P. crustosum, Penicillium crustosum; M. madagascariense, Mycobacterium madagascariense; M. indicus pranii, Mycobacterium indicus pranii; E. faecium, Enterococcus faecium.*

Phytochemistry of Zanthoxylum chalybeum

A variety of phytochemical constituents have been isolated from *Z. chalybeum* root barks, stem barks, leaves and seeds. Generally, *Z. chalybeum* contains tannins, reducing sugars, saponins, alkaloid salts, anthraenosides, flavinosides, steroidglycosides triterpenes and anthocyanosides (Nalule et al. 2013). A detailed phytochemical analysis of essential oils in *Z. chalybeum* revealed the presence of neral, limonene, geranial and terpinene-4-ol (Chagonda et al. 1994; Chisowa et al. 1999; Ocheng et al. 2015). However, 3-careen, 4-careen,

TABLE 3: Classes of compounds isolated from *Zanthoxylum chalybeum.*

Chemical	Molecular formula	Plant part	Country	Reference
3-(1-isopenoloxy)-4-methoxyfagaamide	$C_{19}H_{27}O_3N$	Stem bark	Kenya	Gacheru (2018)
Fagaramide	$C_{14}N_{17}NO_3$	Stem bark	Kenya	Gacheru (2018)
Sesamin	$C_{20}H_{18}O_6$	Stem, root bark	Kenya	Gacheru (2018); Ochieng et al. (2020)
3-Carene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
4=Carene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
Cis-β-ocimene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
α-Phellandrene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
β-Phellandrene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
α-Pinene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
β-Pinene	$C_{10}H_{16}$	Leaves	Uganda	Ocheng et al. (2015)
Geraniol	$C_{10}H_{18}O$	Leaves	Uganda	Ocheng et al. (2015)
Geranyl acetate	$C_{12}H_{20}O_2$	Leaves	Uganda	Ocheng et al. (2015)
6-methyl-5-hepten-2-one	$C_8H_{14}O$	leaves	Zimbabwe	Chagonda et al. (1994)
Linalool	$C_{10}H_{18}O$	Leaves	Uganda	Ocheng et al. (2015)
Cis-β-terpineol	$C_{10}H_{18}O$	Leaves	Uganda	Ocheng et al. (2015)
Decanal	$C_{10}H_{20}O$	Leaves	Uganda	Ocheng et al. (2015)
Terpinene-4-ol,	$C_{10}H_{18}O$	Leaves	Uganda Zambia Zimbabwe	Ocheng et al. (2015); Chisowa et al. (1999); Chagonda et al. (1994)
Dihydrochelerythrine	$C_{21}H_{19}NO_4$	Roots	Ethiopia	Anza et al. (2014)
Skimmianine	$C_{14}H_{13}NO_4$	Seeds, root bark	Uganda, Kenya	Olila et al. (2001); Ochieng et al. (2020)
Neryl acetate	$C_{12}H_{20}O_2$	Leaves	Zimbabwe	Chagonda et al. (1994)
N-methylcorydine	$C_{21}H_{26}NO_4^+$	Root bark	Kenya	Fish and Watermann (1972b)
Berberine	$C_{20}H_{18}NO_4^+$	Root bark	Kenya	Fish and Watermann (1972b)
Limonene	$C_{10}H_{16}$	Leaves	Zambia Zimbabwe Uganda	Chisowa et al. (1999); Chagonda et al. (1994); Ocheng et al. (2015)
Neral	$C_{10}H_{16}O$	Leaves	Zambia Zimbabwe Uganda	Chisowa et al. (1999); Chagonda et al. (1994); Ocheng et al. (2015)
Candicine	$C_{11}H_{18}NO^+$, $HOC_6H_4(CH_2)_2N(CH_3)_3^+$	Root bark	Zimbabwe	Fish and Waterman (1972b)
Tembetarine	$C_{20}H_{26}NO_4^+$	Root bark	Zimbabwe	Fish and Watermann (1972b)
Magnoflorin	$C_{20}H_{24}NO_4^+$	Root bark	Zimbabwe	Fish and Watermann (1972b)
Geranial	$C_{10}H_{16}O$	Leaves	Zambia Zimbabwe Uganda	Chisowa et al. (1999); Chagonda et al. (1994); Ocheng et al. (2015)
Terpinolene	$C_{10}H_{16}$	Leaves	Uganda Zimbabwe	Chagonda et al. (1994); Ocheng et al. (2015)
Linalyl propionate	$C_{13}H_{22}O_2$	Leaves	Zimbabwe	Chagonda et al. (1994)
Chelerythrine	$C_{21}H_{18}NO_4^+$	Root bark	Kenya	Fish and Watermann (1972a)
Nitidine	$C_{21}H_{18}NO_4^+$	Root bark	Kenya	Fish and Watermann (1972a)
Citronella	$C_{10}H_{18}O$	Leaves	Uganda Zimbabwe	Chagonda et al. (1994); Ocheng et al. (2015)
Camplenen	$C_{10}H_{16}$	Leaves	Zimbabwe	Chagonda et al. (1994)
1.8 cineole	$C_{10}H_{18}O$	Leaves	Uganda Zambia	Chisowa et al. (1999); Ocheng et al. (2015)
Sabinen	$C_{10}H_{16}$	Leaves	Zambia	Chisowa et al. (1999)
Trans-p-menth-2-en-1-ol	$C_{10}H_{18}O$	Leaves	Zambia	Chisowa et al. (1999)
α Terpineol	$C_{10}H_{18}O$	Leaves	Zambia Uganda	Chisowa et al. (1999); Ocheng et al. (2015)
6-Benzo [1, 3] dioxol-5-yl-hexa-2,5 dienoic acid isobutylamide (Chalybemide A)	$C_{13}H_{15}NO_3$	Root bark	Kenya	Ochieng et al. (2020)
4-Methoxy-N-(2-methoxy-phenyl)-Nmethyl-benzamide (Chalybemide B)	$C_{17}H_{21}NO_3$	Root bark	Kenya	Ochieng et al. (2020)
N-(2-Hydroxy-methyl-propyl)-3 phenyl-acrylamide (Chalybemide C)	$C_{17}H_{19}NO_3$	Root bark	Kenya	Ochieng et al. (2020)
Trans-fagaramide	$C_{14}H_{17}NO_3$	Root bark	Kenya	Ochieng et al. (2020)
Norchelerythrine	$C_{20}H_{15}NO_4$	Root bark	Kenya	Ochieng et al. (2020)
6-Acetonyldihydrochelerythrine	$C_{23}H_{21}NO_5$	Root bark	Kenya	Ochieng et al. (2020)
6-Hydroxy-*N*-methyl decarine	$C_{10}H_{12}N_2O$	Root bark	Kenya	Ochieng et al. (2020)
Ailanthoidol	$C_{19}H_{18}O_5$	Root bark	Kenya	Ochieng et al. (2020)
Lupeol	$C_{30}H_{50}O$	Root bark	Kenya	Ochieng et al. (2020)
Lupanone	$C_{30}H_{50}O$	Root bark	Kenya	Ochieng et al. (2020)
20-Hydroxy-3-oxo-28-lupanoic acid	$C_{30}H_{48}O_4$	Root bark	Kenya	Ochieng et al. (2020)
3α,20,28-Trihydroxylupane	$C_{30}H_{50}0_3$	Root bark	Kenya	Ochieng et al. (2020)

Note: Please see the full reference list of the article, Mguni, S, Mashinya, F, Khabo-Mmekoa, C. & Shai, L.J., 2023, 'A review of Zanthoxylum chalybeum Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicologyl.', *Journal of Medicinal Plants for Economic Development* 7(1), a202. https://doi.org/10.4102/jomped.v7i1.202, for more information.

Cis-β-Ocimene, β-Phellandrene, α- Phellandrene, α-Pinene, β-Pinene, geraniole, geranyl acetate, linalool, Cis-β-terpineol and decanal were only present in *Z. chalybeum* plants that are found in Uganda (Ocheng et al. 2015). Neryl acetate, linalyl propionate and camphene were only found in Zimbabwe (Chagonda et al. 1994) and sabinen, and

TABLE 4: Toxicological properties of *Zanthoxylum chalybeum* in reviewed studies.

Plant part	Compound or Extract	Model of experimentation	Findings	Author
Root bark	Ethanol acetic acid 9:1	Albino Wister rats	No death or remarkable changes after administration	Engeu et al. (2008)
Stem bark	Sesamin, 3-(1-isopenoloxy)-4-methoxyfagaamide and fagaramide	Resazurin test on drug-sensitive and MDR leukaemia cell lines	All exhibited < 70% of viable cells at 10 µg/mL	Gacheru (2018)
Leaves and twigs	Tepinene-4-ol,	MTT on human gingival fibroblast cell line	IC_{50} 26 µg/mL	Ocheng et al. (2016)
Root bark,	Methanol	Brine shrimp	LL_{50} of 68.9 µg/mL	Moshi et al. (2007)
Leaves	Dichloromethane and Methanol	Resazurin on human leukaemia cell (HL 60) and *T B brucei* cell lines	*T b brucei* – methanol IC_{50} of 36 µg/mL and DCM 11 µg/mL HL 60 – methanol 137 µg/mL and DCM 30 µg/mL	Nibret et al. (2009)
Root bark	Dichloromethane and Methanol	Lactate dehydrogenase assay on 3D7 (chloroquine sensitive) and W2 (chloroquine resistant) *P. falciparum*	Methanol – (3D7)- 4.2 µg/mL, (W2) 1.9 µg/mL DCM – (3D7) – 6.2 µg/mL	Muganga et al. (2010)
Stem bark,	Dichloromethane	Brine shrimp	LC_{50} of 5.74 µg/mL	Chrian et al. (2011)
Stem bark, root bark	Dichloromethane: Chloroform	MTT on Vero99 cells	Both had CC_{50} of >100 µg/mL	Njenga et al. (2016)
Stem bark	4-(Isoprenyloxy)-3-methoxy,4-deoxymethylenedioxyfagaramide	Resazurin reduction assay on CCRF-CEM (drug sensitive) and CEM/ADR 5000 (drug resistant) leukaemia cell lines	IC_{50} of 29.13 ± 2.54 µM on drug-sensitive CCRF-CEM and IC_{50} of 31 ± 4.74 µM on drug-resistant CEM/ADR5000 leukaemia cell lines.	Omosa et al. (2021)
Leaf	Methanol, Dichloromethane, Aqueous extracts	MTT on D6 (chloroquine sensitive) and W6 (chloroquine resistant) *Plasmodium falciparum*	Aqueous – 103.19 ± 8.01 on D6 and 107.44 + 7.28 on W6 cells. DCM – 33.47 ± 6.78 on D6 and 36.25 ± 8.87 on W6 cells. Methanol – 26.068 ±7.50 on D6 and 39.26 ± 6.80 on W6 cells	Waiganjo et al. (2020)
Leaf, root bark, stem bark	$CHCl_3$ / MeOH, 1:1	Brine shrimp	LC_{50}: Leaves – 62 µg/ml Stem bark – 19 µg/mL, Root bark – 11 µg/ml	Nguta et al. (2011)
Leaf, root bark, stem bark	Aqueous	Brine shrimp	LC_{50}: Leaves – 31 µg/ml Stem bark – 288 µg/mL, Root bark – 56 µg/ml	Nguta et al. (2011)
Stem bark	Diethyl ethanol	Lactate dehydrogenase assay on human keratinocyte cell line (HaCaTs cells)	IC_{50} > 515 µg/mL	Schultz et al. (2020)
Root bark and stem bark	Methanol: dichloromethane (1:1)	Brine shrimp	LC_{50} of 38.5 µg/mL (root bark) and 26.3 µg/mL (stem bark)	Matata et al. (2018)
Stem bark	Aqueous and Chloroform: methanol (1:1)	Brine shrimp	LD_{50} – Aq of 268.28 µg/mL and chloroform: methanol (1:1) of 25.78 µg/mL.	Musila (2012)
Stem bark	Diethyl ethanol	Human keratinocyte toxicity assay on human keratinocytes (HaCaT)	IC_{50} < 512 µg/mL	Schultz et al. (2020)

Note: Please see the full reference list of the article, Mguni, S, Mashinya, F, Khabo-Mmekoa, C. & Shai, L.J., 2023, 'A review of Zanthoxylum chalybeum Engl: Ethnomedicinal uses, pharmacology, phytochemistry and toxicologyl.', *Journal of Medicinal Plants for Economic Development* 7(1), a202. https://doi.org/10.4102/jomped.v7i1.202, for more information.

MDR, multi drug resistant.

trans-p-menth-2-en-1-ol was isolated from Zambian samples (Chisowa et al. 1999). Omosa et al. (2021) isolated a new compound, 4-(isoprenyloxy)-3-methoxy,4-deoxymethylenedi oxyfagaramide, from the $MeOH/CH_2Cl_2$ (1:1) extract of *Z. chalybeum.* Ochieng et al. (2020) also isolated 6-benzo (1, 3) dioxol-5-yl-hexa-2,5 dienoic acid isobutylamide, 4-methoxy-N-(2-methoxy-phenyl)-N methyl-benzamide and N-(2-hydroxy-methyl-propyl)-3 phenyl -acrylamide from the methanolic root bark extract of *Z. chalybeum*. Other noteworthy compounds identified include sesamin, 3-(1-isopenoloxy)-4-methoxyfagaramide and fagaramide (Gacheru 2018), 2 3-epoxy 6, 7-methylenedioxy coniferylalcohol, dihydrochelerythrine (Anza et al. 2014), N-isobutyl-3-(3, 4-methylene dioxyphenyl)-2E-propenamide (Adia et al. 2016) and skimianine (Olila, Olwa-Odyek & Opuda-Asibo 2001).

Toxicology of Zanthoxylum chalybeum

Analysis of toxicity studies on *Z. chalybeum* shows that the stem bark has the most potent toxicity compared to other plant parts. The stem and root bark extracts exhibited highly potent toxicity using the Brine shrimp lethality (BSL) assay. Although the $CHCl_3$/methanol 1:1 root bark extract exhibited a high toxicity on BSL (11 µg/mL) (Nguta et al. 2011), Matata et al. (2018) exhibited moderate (38.5 µg/mL) toxicity, and this could be attributed to differences in geographical locations that contribute to differences in phytochemical constituents. Aqueous extracts exhibited mostly low to moderate potency (Nguta et al. 2011; Waiganjo et al. 2020) from all plant parts. Adia et al. (2016) and Gacheru (2018) isolated fagaramide from stem barks of *Z. chalybeum* and both exhibited potent toxicity on both leukaemia and *P. falciparum* cell lines. In other studies, Sesamin, 3-(1-isopenoloxy)-4 methoxyfagaamide and 4-(isoprenyloxy)-3-methoxy, 4-deoxymethylenedioxyfagaramide isolated from stem barks of *Z. chalybeum* exhibited significant toxicity on both sensitive and resistant leukaemia and *P. falciparum* cell lines (Omosa et al. 2021; Gacheru 2018), and these could attribute to the high levels of toxicity in stem barks.

Implications and recommendations

Herbal medicines have been used in medical practice for many years and have contributed immensely to the maintenance of human health especially in developing countries. Scientific research on *Z. chalybeum* suggests a huge biological potential of the plant. The most common plant part that is used by traditional medical practitioners is the root bark. Kitula (2007) reported that roots and stems contain high concentrations of active compounds than other plant parts. However, high utilisation of these parts may harm their sustainability, unless proper harvesting techniques are implemented (Mbinile et al. 2020). Although *Z. chalybeum* is abundantly distributed in East,

West and Central Zimbabwe and is not listed on the red data list of the threatened plant species in Zimbabwe, such actions may jeopardise its conservation efforts. The widespread utilisation of the plant's roots can have negative consequences as it can result in the complete depletion of the plant. This, in turn, may pose a risk to the species, making it increasingly rare, endangered or possibly extinct.

Zanthoxylum chalybeum has displayed a wide range of pharmacological activities in Eastern and Southern Africa. Ochieng et al (2020) established that alkaloids from *Z.chalybeum* root barks played a significant role in inhibiting α-amylase and β-glucosidase activities. Their results confirmed the antihyperglycemic potential of alkaloids from *Z. chalybeum*, which lends credence to its use towards diabetes susceptibilities. Kaigongi et al. (2022) recently demonstrated that there is a correlation between antimicrobial and antioxidant activity in *Z. chalybeum*. They showed that the lower the DPPH value for antioxidant activity, the higher the antimicrobial activity. Despite most researchers showing inhibition of pathogenic organisms (Kaigongi et al. 2014; Maima & Muyendo 2018), some did not show any inhibition (Nguta & Kiraithe 2019; Olila, Olwa-Odyek & Opuda-Asibo 2001). It should be noted that traditional medicine involves the use of combinations of plants in the form of decoctions and infusions which use water as a solvent. These combinations may help activate inactive chemicals or may have a stronger synergistic effect when combined. An alternative explanation is that extracts from Z. *chalybeum* may exert their effects indirectly, and it is possible that the active components may exist as precursors necessitating activation within the body through unknown mechanisms (Olila, Olwa-Odyek and Opuda-Asibo 2001).

The qualitative phytochemical analysis of *Z. chalybeum* still needs to be exhausted. Although essential oils of *Z. chalybeum* have been analysed in three countries in Africa (Chagonda et al. 1994; Chisowa et al. 1999; Ocheng et al. 2015), more research is still needed in order to determine the constituents of other phytochemicals.

This could prove valuable for scientists and researchers seeking to identify novel chemical compounds responsible for the reputed applications of the plant. Differences in phytochemical quality and quantity could be attributed to climatic differences, seasonal differences, soil and the age of plants. The solvent used may also play a significant role in determining the chemicals extracted. It is known that phenolic compounds, flavonoids and alkaloids are easily extracted in high amounts by polar solvents such as methanol, ethanol, ethyl acetate and aqueous solvents (Iloki-Assanga et al. 2015). Non-polar solvents such as acetone, n-hexane, petroleum ether and diethyl ether are known to extract chemicals like liphophilic compounds including alcanas, waxes, colour pigments, sterols, several terpenoids and some alkaloids.

Herbal medicines have demonstrated efficacy in treating a range of infections, yet their safety has often been neglected. Although there are a few studies on phytochemical analysis and toxicity, *Z. chalybeum* extracts and phytochemicals may generally be toxic especially in higher concentrations, and this may be attributed by unknown compounds. The time of collection has a significant impact on the cytotoxicity of the plant. Muganga et al. (2010) collected samples, during the dry and rainy seasons. The samples collected during the dry season were less active (IC_{50} 38.3 µg/mL) than those collected during the rainy season (IC_{50} 4.2 µg/mL), suggesting that the rainy season is appropriate for the synthesis of active ingredients. Differences in toxicity assays may produce different results. Although the BSL assay is not a reliable method of testing toxicity because of the movement of live cells, which may result in wrongful counting, it could serve as a baseline for evaluating and contrasting the cytotoxic effects of different plants. There is, therefore, a need to standardise the toxicological properties of *Z. chalybeum* and their detailed clinical trials.

Conclusion

This review has established that *Z. chalybeum* is a valued medicinal plant species used by several local communities in East and Southern Africa. Despite its wide range of ethnomedicinal applications, more scientific evaluation still needs to be done to merge traditional and folkloric claims with scientific knowledge.

An understanding of *Z. chalybeum* phytochemicals and the determination of toxicities of potential phytochemicals is inevitable. The inhibition of the growth of ESKAPE pathogens by *Z. chalybeum* root barks is a major breakthrough that calls for more investigations of the plant as multi-drug resistant pathogens are a global concern. No studies have also been done to understand the molecular mechanisms of how *Z. chalybeum* phytochemicals lead to the death of these pathogens.

Acknowledgements

The authors would like to thank the Tshwane University of Technology for all the technical support.

Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

F.M. conceived the main conceptual ideas and proof outline. S.M. collected the data and wrote the manuscript with support and supervision from F.M., and L.J.S. and C.K-M. were in consultation and supervision.

Ethical considerations

This article does not contain any studies involving human participants performed by any of the authors. Ethical clearance

waiver received from the Ethics Committee-Faculty Committee for Research Ethics-Science (FCRE-SCI) Tshwane University of Technology FCRE 2022/05/002 (SCI) (FCPS 01).

Funding information

This research received no specific grant from any funding agency in the public, commercial or not-for- profit sectors.

Data availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

References

Adia, M.M., Emami, S.N., Byamukama, R., Faye, I. & Borg-Karlson, A.K., 2016, 'Antiplasmodial activity and phytochemical analysis of extracts from selected Ugandan medicinal plants', *Journal of Ethnopharmacology* 186, 14–19. https://doi.org/10.1016/j.jep.2016.03.047

Agwaya, M.S. & Nandutu, A.M., 2016, 'Hypoglycemic activity of aqueous root bark extract *Zanthoxylum chalybeum* in alloxan-induced diabetic rats', *Journal of Diabetes Research* 2016, 1–5. https://doi.org/10.1155/2016/8727590

Agwaya, M.S., Nandutu, A.M. & Vuzi, P.C., 2016, 'Protective effects of *Zanthoxylum chalybeum* in diabetes-induced myocardial dysfunction in rats', *European Journal of Medicinal Plants* 12(1), 1–10. https://doi.org/10.9734/EJMP/2016/22137

Anza, M., Haile, E., Tadesse, S., Mammo, F. & Endale, M., 2014, 'A coniferyl alcohol derivative from the roots of *Zanthoxylum chalybeum*', *Journal of Coastal Life Medicine* 2(12), 970–974.

Bbosa, G.S., Mwebaza, N., Lubega, A., Musisi, N., Kyegombe, D.B. & Ntale, M., 2014, 'Antiplasmodial activity of leaf extracts of *Zanthoxylum chalybeum* Engl', *British Journal of Pharmaceutical Research* 4(6), 705–713. https://doi.org/10.9734/BJPR/2014/6528

Chagonda, L., Gundidza, M., Wijesuria, A.M.S. & Mavi, S., 1994, 'Composition of the leaf oil of *Zanthoxylum chalybeum* Engl', *Journal of Essential Oil Research* 6(6), 561–563. https://doi.org/10.1080/10412905.1994.9699346

Chhabra, S.C., Mahunnah, R.L.A. & Mshiu, E.N., 1990, 'Plants used in traditional medicine in Eastern Tanzania. IV. Angiosperms (Mimosaceae to Papilionaceae)', *Journal of Ethnopharmacology* 29(3), 295–323. https://doi.org/10.1016/0378-8741(90)90041-Q

Chisowa, E.H., Hall, D.R. & Farman, D.I., 1999, 'Volatile constituents of the leaf oil of *Zanthoxylum chalybeum* Engl', *Journal of Essential Oil Research* 11(3), 360–362. https://doi.org/10.1080/10412905.1999.9701154

Chrian, M., Erasto, P. & Otieno, N.J., 2011, 'Antimycobacterial activity and cytotoxicity effect of extracts of *Hallea rubrostipulata* and *Zanthoxylum chalybeum*', *Spatula DD* 1(3), 147–152. https://doi.org/10.5455/spatula.20110811025348

Engeu, O.P., Tumusiime, R., Agwaya, M., Mugisha, G., Nambatya Kyeyune, G., Galiwango, B. et al., 2008, 'Repeat-dose effects of *Zanthoxylum chalybeum* root bark extract: A traditional medicinal plant used for various diseases in Uganda', *African Journal of Pharmacy and Pharmacology* 2(6), 101–105.

Fish, F. & Waterman, P.G., 1972a, 'Lirioresinol-B-dimethyl ether from the bark of *Fagara leprieurii*', *Phytochemistry* 11(4), 1527–1528. https://doi.org/10.1016/S0031-9422(00)90138-9

Fish, F. & Waterman, P.G., 1972b, 'Methanol-soluble quaternary alkaloids from African Fagara species', *Phytochemistry* 11(10), 3007–3014. https://doi.org/10.1016/0031-9422(72)80094-3

Gacheru, M.M., 2018, 'Phytochemical investigation of Zanthoxylum Paracanthum and Zanthoxylum chalybeum for cytotoxic principles against drug sensitive and multidrug resistant Leukemia cancer cell lines', Doctoral dissertation, University of Nairobi.

Hedberg, I., Hedberg, O., Madati, P.J., Mshigeni, K.E., Mshiu, E.N. & Samuelsson, G., 1983, 'Inventory of plants used in traditional medicine in Tanzania. Part III. Plants of the families Papilionaceaevitaceae', *Journal of Ethnopharmacology* 9(2–3), 237–260. https://doi.org/10.1016/0378-8741(83)90034-X

Iloki-Assanga, S.B., Lewis-Luján, L.M., Lara-Espinoza, C.L., Gil-Salido, A.A., Fernandez-Angulo, D., Rubio-Pino, J.L. et al., 2015, 'Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum*', *BMC Research Notes* 8(1), 1–14. https://doi.org/10.1186/s13104-015-1388-1

Jolly, R., Jefwa, J. & Mwafaida, J., 2017, 'Validation of the ability of selected medicinal plants to control bacterial diseases as applied by local herbalists in Kaya Tsolokero and Kaya Kauma of Kenya', *Forest Research: Open Access* 6(2), 1–7.

Kaigongi, M.M., Dossaji, S., Nguta, J., Lukhoba, C. & Musila, F., 2014, 'Antimicrobial activity, toxicity and phytochemical screening of four medicinal plants traditionally used in Msambweni district, Kenya', *Journal of Biology, Agriculture and Healthcare* 4(28), 6–12.

Kaigongi, M.M., Lukhoba, C.W., Musila, F.M., Taylor, M., Mbugua, R.W., Githiomi, J. et al., 2022, 'A versatile untargeted metabolomics-driven technology for rapid phytochemical profiling of stem barks of *Zanthoxylum* species with antioxidant and antimicrobial activities', *Advances in Traditional Medicine* 1–17. https://doi.org/10.1007/s13596-022-00676-w

Kimani, C.N., Mbaria, J.M., Suleiman, M., Gakuya, D. & Kiama, S.G., 2015, 'Antihyperglycemic activity of *Zanthoxylum chalybeum* stem bark extracts in diabetic rats', *Journal of Phytopharmacology* 4(3), 183–189. https://doi.org/10.31254/phyto.2015.4310

Kipkore, W., Wanjohi, B., Rono, H. & Kigen, G., 2014, 'A study of the medicinal plants used by the Marakwet Community in Kenya', *Journal of Ethnobiology and Ethnomedicine* 10(1), 1–22. https://doi.org/10.1186/1746-4269-10-24

Kiraithe, M.N., Nguta, J.M., Mbaria, J.M. & Kiama, S.G., 2016, 'Evaluation of the use of *Ocimum suave* Wild. (Lamiaceae), *Plectranthus barbatus* Andrews (Lamiaceae) and *Zanthoxylum chalybeum* Engl. (Rutaceae) as antimalarial remedies in Kenyan folk medicine', *Journal of Ethnopharmacology* 178, 266–271. https://doi.org/10.1016/j.jep.2015.12.013

Kiringe, J.W., 2006, 'A survey of traditional health remedies used by the Maasai of Southern Kaijiado District, Kenya', *Ethnobotany Research and Applications* 4, 61–73. https://doi.org/10.17348/era.4.0.61-74

Kitula, R.A., 2007, 'Use of medicinal plants for human health in Udzungwa Mountains Forests: A case study of New Dabaga Ulongambi Forest Reserve, Tanzania', *Journal of Ethnobiology and Ethnomedicine* 3(1), 1–4. https://doi.org/10.1186/1746-4269-3-7

Kokwaro, J.O., 2009, *Medicinal plants of east Africa*, University of Nairobi press, Nairobi.

Kokwaro, J.O., 1993, 'Medicinal plants of East Africa. Published and printed by Kenya literature bureau', *Nairobi Kenya* 62(2), 176–177.

Mahamadi, C. & Wunganayi, T., 2018, 'Green synthesis of silver nanoparticles using *Zanthoxylum chalybeum* and their antiprolytic and antibiotic properties', *Cogent Chemistry* 4(1), 1–12. https://doi.org/10.1080/23312009.2018.1538547

Maima, A.O. & Munyendo, W., 2019, 'Antimicrobial assay of aqueous extracts of selected ethno-pharmacologic alternatives used by the Maasai community of Narok, Kenya', *European Journal of Medicinal Plants* 26(3), 1–11. https://doi.org/10.9734/EJMP/2018/46227

Matata, D.Z., Ngassapa, O.D., Machumi, F. & Moshi, M.J., 2018, 'Screening of plants used as traditional anticancer remedies in mkuranga and same districts, Tanzania, using brine shrimp toxicity bioassay', *Evidence-Based Complementary and Alternative Medicine* 2018, 3–13. https://doi.org/10.1155/2018/3034612

Mbinile, S.D., Munishi, L.K., Ngondya, I.B. & Ndakidemi, P.A., 2020, 'Conservation and management challenges facing a medicinal plant *Zanthoxylum chalybeum* in Simanjiro area, Northern Tanzania', *Sustainability* 12(10), 4140–4151. https://doi.org/10.3390/su12104140

Medhi, K., Deka, M. & Bhau, B.S., 2013, 'The genus *Zanthoxylum* – A stockpile of biological and ethnomedicinal properties', *Open Access Scientific Reports* 2(3), 1–8.

Mollel, J.T., Said, J.S., Masalu, R.J., Hannoun, C., Mbunde, M.V., Nondo, R.S. et al., 2022, 'Anti-respiratory syncytial virus and anti-herpes simplex virus activity of six Tanzanian medicinal plants with extended studies of *Erythrina abyssinica* stem bark', *Journal of Ethnopharmacology* 292, 1–11. https://doi.org/10.1016/j.jep.2022.115204

Moshi, M.J. & Mbwambo, Z.H., 2002, 'Experience of Tanzanian traditional healers in the management of non-insulin dependent diabetes mellitus', *Pharmaceutical Biology* 40(7), 552–560. https://doi.org/10.1076/phbi.40.7.552.14691

Moshi, M.J., Van den Beukel, C.J., Hamza, O.J., Mbwambo, Z.H., Nondo, R.O., Masimba, P.J. et al., 2007, 'Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections', *African Journal of Traditional, Complementary and Alternative Medicines* 4(2), 219–225. https://doi.org/10.4314/ajtcam.v4i2.31211

Muganga, R., Angenot, L., Tits, M. & Frederich, M., 2010, 'Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria', *Journal of Ethnopharmacology* 128(1), 52–57. https://doi.org/10.1016/j.jep.2009.12.023

Musila, F.M., 2012, *In vivo antimalarial activity, toxicity and phytochemical screening of aqueous and organic extracts of selected antimalarial plants in Msambweni district, Kenya*, Doctoral dissertation, University of Nairobi.

Nalule, A.S., Mbaria, J.M. & Kimenju, J.W., 2013, 'In vitro anthelmintic potential and phytochemical composition of ethanolic and water crude extracts of *Euphorbia heterophylla* Linn', *African Journal of Pharmacy and Pharmacology* 7(23), 1605–1614. https://doi.org/10.5897/AJPP2013.3664

Neuwinger, H.D., 1996, *African ethnobotany: Poisons and drugs: Chemistry, pharmacology, toxicology*, Chapman and Hall/CRC Press, Boca Raton, FL.

Nguta, J.M. & Kiraithe, M.N., 2019, 'In vitro antimicrobial activity of aqueous extracts of *Ocimum suave* Willd., *Plectranthus barbatus* Andrews and *Zanthoxylum chalybeum* Engl. against selected pathogenic bacteria', *Biomedical and Biotechnology Research Journal (BBRJ)* 3(1), 30–34. https://doi.org/10.4103/bbrj.bbrj_128_18

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kabasa, J.D. & Kiama, S.G., 2011, 'Biological screening of Kenya medicinal plants using *Artemia salina* L. (Artemiidae)', *Pharmacologyonline* 2(1), 458–478. https://doi.org/10.4081/dts.2012.e12

Nibret, E., Ashour, M.L., Rubanza, C.D. & Wink, M., 2010, 'Screening of some Tanzanian medicinal plants for their trypanocidal and cytotoxic activities', *Phytotherapy Research* 24(6), 945–947. https://doi.org/10.1002/ptr.3066

Njenga, D., Irungu, B., Mbaria, J., Mutai, C. & Nguta, J., 2016, 'Antiplasmodial activity, cytotoxicity and acute toxicity of *Zanthoxylum chalybeum* Engl.', *World Journal of Pharmacy and Pharmaceutical Sciences* 5, 208–217.

Njoroge, G.N. & Bussmann, R.W., 2006, 'Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya)', *Journal of Ethnobiology and Ethnomedicine* 2, 1–7. https://doi.org/10.1186/1746-4269-2-8

Nyongesa, D.W., 2019, 'Evaluation of A-Amylase and A-Glucosidase Inhibitory compounds from Zanthoxylum chalybeum stem and root bark', Doctoral dissertation, Maseno University.

Ocheng, F., Bwanga, F., Almer Boström, E., Joloba, M., Borg-Karlson, A.K., Yucel-Lindberg, T. et al., 2016, 'Essential oils from Ugandan medicinal plants: in vitro cytotoxicity and effects on IL-1β-induced proinflammatory mediators by human gingival fibroblasts', *Evidence-Based Complementary and Alternative Medicine* 2016, 1–8. https://doi.org/10.1155/2016/5357689

Ocheng, F., Bwanga, F., Joloba, M., Softrata, A., Azeem, M., Pütsep, K. et al., 2015, 'Essential oils from Ugandan aromatic medicinal plants: Chemical composition and growth inhibitory effects on oral pathogens', *Evidence-Based Complementary and Alternative Medicine* 2015, 1–10. https://doi.org/10.1155/2015/230832

Ochieng, C.O., Nyongesa, D.W., Yamo, K.O., Onyango, J.O., Langat, M.K. & Manguro, L.A., 2020, 'α-Amylase and α-glucosidase inhibitors from *Zanthoxylum chalybeum* Engl. root bark', *Fitoterapia* 146, 1–7. https://doi.org/10.1016/j.fitote.2020.104719

Olila, D., Olwa-Odyek & Opuda-Asibo, J., 2002, 'Screening of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis* for activity against measles virus (Swartz and Edmonston strains) in vitro', *African Health Sciences* 2(1), 2–10.

Olila, D., Olwa-Odyek & Opuda-Asibo, J., 2001, 'Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis,* Ugandan medicinal plants', *African Health Sciences* 1(2), 66–72.

Omara, T., 2020, 'Antimalarial plants used across Kenyan communities', *Evidence-Based Complementary and Alternative Medicine* 2020, 1–31. https://doi.org/10.1155/2020/4538602

Omosa, L.K., Mbogo, G.M., Korir, E., Omole, R., Seo, E.J., Yenesew, A. et al., 2021, 'Cytotoxicity of fagaramide derivative and canthin-6-one from *Zanthoxylum* (Rutaceae) species against multidrug resistant leukemia cells', *Natural Product Research* 35(4), 579–586. https://doi.org/10.1080/14786419.2019.1587424

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Simons, A., 2009, *Agroforestree Database: A tree reference and selection guide version 4,* World Agroforestry Center, Nairobi.

Pierre, M.J., Munyabuhoro, S. & Emmanuel, B.S., 2013, 'In vitro antimicrobial investigation of *Zanthoxylum chalybeum* stem bark', *Journal of Medicinal Plants Research* 7(21), 1577–1579.

Rukunga, G.M., Gathirwa, J.W., Omar, S.A., Muregi, F.W., Muthaura, C.N., Kirira, P.G. et al., 2009, 'Anti-plasmodial activity of the extracts of some Kenyan medicinal plants', *Journal of Ethnopharmacology* 121(2), 282–285. https://doi.org/10.1016/j.jep.2008.10.033

Schultz, F., Anywar, G., Tang, H., Chassagne, F., Lyles, J.T., Garbe, L.A. et al., 2020, 'Targeting ESKAPE pathogens with anti-infective medicinal plants from the Greater Mpigi region in Uganda', *Scientific Reports* 10(1), 1–19. https://doi.org/10.1038/s41598-020-67572-8

Tabuti, J.R., 2008, 'Herbal medicines used in the treatment of malaria in Budiope county, Uganda', *Journal of Ethnopharmacology* 116(1), 33–42. https://doi.org/10.1016/j.jep.2007.10.036

Tabuti, J.R., Kukunda, C.B. & Waako, P.J., 2010, 'Medicinal plants used by traditional medicine practitioners in the treatment of tuberculosis and related ailments in Uganda', *Journal of Ethnopharmacology* 127(1), 130–136. https://doi.org/10.1016/j.jep.2009.09.035

Taniguchi, M., Chapya, A., Kubo, I. & Nakanishi, K., 1978, 'Screening of East African plants for antimicrobial activity. I', *Chemical and Pharmaceutical Bulletin* 26(9), 2910–2913. https://doi.org/10.1248/cpb.26.2910

Tuasha, N., Petros, B. & Asfaw, Z., 2018a, 'Plants used as anticancer agents in the Ethiopian traditional medical practices: a systematic review', *Evidence-Based Complementary and Alternative Medicine: eCAM* 2018, 1–28. https://doi.org/10.1155/2018/6274021

Tuasha, N., Petros, B. & Asfaw, Z., 2018b, 'Medicinal plants used by traditional healers to treat malignancies and other human ailments in Dalle District, Sidama Zone, Ethiopia', *Journal of Ethnobiology and Ethnomedicine* 14(1), 1–21. https://doi.org/10.1186/s13002-018-0213-z

Waiganjo, B., Moriasi, G., Onyancha, J., Elias, N. & Muregi, F., 2020, 'Antiplasmodial and cytotoxic activities of extracts of selected medicinal plants used to treat malaria in Embu County, Kenya', *Journal of Parasitology Research* 14(1), 1–12.

Wambugu, S.N., Mathiu, P.M., Gakuya, D.W., Kanui, T.I., Kabasa, J.D. & Kiama, S.G., 2011, 'Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya', *Journal of Ethnopharmacology* 137(2), 945–955. https://doi.org/10.1016/j.jep.2011.06.038

World Health Organization (WHO). Programme on Traditional Medicine, 2001, *Legal status of traditional medicine and complementary/alternative medicine: a worldwide review*, World Health Organization, Geneva.