Antimicrobial Properties of Medicinal Plants Against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* Bacteria

*Kiptoo Kipkorir, Wanjohi Benard, Ndegwa John and Maina Margaret
University of Eldoret, P.o. Box 1125, Eldoret Kenya

***Corresponding author's Email address:** kipkorirk@gmail.com

Abstract

Herbal medicine has been used in Africa for centuries and continues to be an important aspect of traditional medicine in many African communities. While Euclea divinorum, Carissa edulis and Prunus africana has a long history of traditional use in Kenya, more research is needed to determine its safety and efficacy for these medicinal purposes. Therefore, this research studied the antimicrobial capabilities of Euclea divinorum Hern (Ebenaceae), Carissa edulis, and Prunus africana against Staphylococcus aureus, Escherichia coli, and Candida albicans bacteria to complement the work of other researchers. Leaves, roots and stem barks of of the three plants were purposively collected from Elgeyo Marakwet County. The samples were analyzed at University of Eldoret Biotechnology Laboratory, Kenya. The samples were ground into powder and successively extracted with hexane, methanol and acetone. Antimicrobial activity of the extracts was determined by agar disc diffusion method. After 24 hours of introducing the roots, leaves and stem bark extracts to the colonies on petri dishes, the inhibitory diameters of the wells were measured to test their antibacterial activity. The roots, leaves and stem bark extracts of E. divinorum, C. edulis and P. africana against S. aureus, E. coli, and C. albicans exhibit varying degrees of antimicrobial activities against S. aureus and E. coli bacterial strain and C. albicans fungal strain. E. divinorum and C. edulis roots extracts exhibited antimicrobial potency against S. aureus, E. coli, and C. albicans while the leaves of E. divinorum and P. africana showed antimicrobial activity against S. aureus and E. coli bacterial strain. Lastly, the methanol stem bark extract of P. africana was only active against E. coli, and C. albicans however, the stem bark extract of E. divinorum and C. edulis were not against S. aureus, E. coli, and C. albicans. It is therefore recommended that root extracts of E. divinorum and C. edulis and the stem bark extracts of P. Africana may provide potential sources for the development of alternative antibacterial agents while E. divinorum and C. edulis agents may provide potential sources for further development of antifungal agents for the treatment of diseases.

Keywords: Herbal plants, antimicrobial activity, bacteria, fungus, *Euclea divinorum*, *Carissa edulis* and *Prunus africana*

INTRODUCTION

Plants have been utilised as medicines for millennia, playing an indispensable role in drug discovery and development (Cragg & Newman, 2001; Zhang et al., 2013; Jachak & Saklani, 2007). The use of medicinal plants can be traced back to ancient times, with evidence of their use in various civilizations such as the Egyptians, Greeks, and Chinese (Jamshidi-Kia et al., 2017; Wadud et al., 2007; Pan et al., 2014; Somvanshi, 2006). Many of these plants were used to treat various ailments and diseases, and some have been found to have therapeutic properties backed by modern scientific research (Hosseinzadeh et al., 2015; Petrovska, 2012; Rahmatullah et al., 2009; Singh, 2015; Rastogi et al., 2016; Oguntibeju, 2018). Throughout history, the use of medicinal plants has evolved and adapted to different cultural and geographical contexts with many traditional remedies still in use to date (Trotter

& Logan, 2019; Alvin et al., 2014).

Herbal medicine has been used in Africa for centuries and continues to be an important aspect of traditional medicine in many African communities (Mahomoodally, 2013; Payyappallimana, 2010). In ancient times, traditional healers used various herbs and plants to treat various ailments, often relying on knowledge passed down from previous generations (Ozioma & Chinwe, 2019; Mahwasane et al., 2013; Che et al., 2017; Adu-Gyamfi & Anderson, 2019). Herbal medicine in Africa is based on a holistic approach that takes into account the physical, mental, and spiritual aspects of health (Gyasi et al., 2016; World Health Organization. 1978). It is often used in combination with other traditional healing practices, such as massage, meditation, and spiritual healing (Okoronkwo et al., 2014; Ezeome & Anarado, 2007). Overall, herbal medicine in Africa remains an important aspect of traditional medicine and continues to be used by many people as a safe and effective alternative to modern medicine. It is estimated that as much as 80% of the African population relies on traditional medicine, including herbal remedies, to meet their healthcare needs (Yuan et al., 2016; Bodeker, 2005). This is partly because the great majority of herbal remedies in African nations are readily available when rural communities have limited access to modern healthcare services (Elujoba et al., 2005; Abdullahi, 2011). Other reasons include cultural beliefs, affordability, sustainability, and efficacy (Kloos et al., 2013). Plants have an abundance of secondary metabolites, including tannins, terpenoids, and alkaloids which have been utilised to treat a wide variety of problems in rural communities, including skin infections, gastrointestinal, respiratory, and gynaecological conditions, among others.

As a developing nation with multiple healthcare issues, such as the high cost of drugs, Kenya must increase its scientific base and develop logical and practical methods to address these issues thogh, few investigations have been conducted on the antimicrobial efficacy of Kenyan herbal plants. *Euclea divinorum*, also known as the "Four-veined Euclea," is a shrub or small tree in the Ebenaceae family (Omara et al., 2022; Nyambe et al., 2021). It is native to southern Africa and can grow up to 5 meters in height (Charles-Dominique et al., 2015). Preliminary phytochemical analyses found previously secondary bioactive substances in many *E. divinorum* parts (Omara et al., 2022). *E. divinorum* root extracts are known to contain polyphenols, saponins, tannins, flavonoids, steroids, terpenoids, glycosides, and alkaloids (Mbabazia et al., 2020; Al-Fatimi, 2019). The methanol extracts of leaves, stems, and fruits include polyphenols and glycosides (Omara et al., 2022). Researchers have found that the root of *E. divinorum* possesses cytotoxic, antibacterial, oxytocic, and diuretic activities. These findings were published by Al-Fatimi et al. (2005), Nyambe (2014), and Kaingu et al (2012). Also, Okello et al. (2010) reported the usage of *Euclea divinorum* to treat snake bites in Kenya. According to Kipkore et al. (2014), *Euclea divinorum* branches are utilised as toothbrushes. There are few reports on antibacterial activity of *Euclea divinorum* in Kenya.

Carissa edulis is a species of flowering plant in the Apocynaceae family, commonly known as the Natal plum or Amatungulu in Zulu (Madani et al., 2017; Ogoma et al.,;2021). *Carissa edulis* is an evergreen shrub or small tree that can reach up to 5 meters (16 feet) tall (Ukwubile et al., 2020). A study done by Ibrahim, (2010) in Norther Nigeria reported that the leaves and fruits extracts of *Carissa edulis* were effective agaist *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Similarly, the aqueous extract of *Carissa edulis* also has demonstrated the potential anti-viral activities at non-cytotoxic concentrations (Tolo et al., 2008). *Carissa edulis* is a medicinal plant that grows particularly in Kenya. The *C. edulis* has been used as a traditional medicine in Kenya for the treatment of various ailments, without any reported side effects. In addition, Opande (2022), while analysing the antibacterial activities of *Carissa edulis* extracts obtained from Kaimosi Forest, Vihiga County, Kenya, observed that the leaves inhibited *Escherichia coli* the most. *Carissa edulis* has also been utilised in Kenya to treat diarrhoea, dysentery, and a variety of skin ailments, including fungal infections and abscesses (Kipkore et al., 2014; Ngiti, 2019).

Prunus africana, also known as African cherry or Pygeum, is a tree species that is native to Africa (Rubegeta et al, 2022; Ingram et al, 2015; Komakech et al, 2022). The *Prunus africana* on the other hand *is* a montane forest species that grows in East and Central Africa (Ingram et al., 2015). Its is an important medicinal plant in traditional African medicine, and its bark extract is used to treat various ailments, including prostate problems, urinary tract infections, and malaria (Komakech & Kang, 2019; Nyamai et al., 2015; Abera, 2014). In Kenya, the tree is found in the montane forests of the eastern and central parts of the country, where it grows at elevations between 1,800 to 3,000 meters (Gladys, 2020). Some of the specific areas where *Prunus africana* can be found in Kenya include: Mount Kenya, Aberdare Ranges and Cherangani Hills (Chebii, 2016). In Kenya, the bark of *Prunus africana* is traditionally used in herbal medicine to treat a variety of ailments, including prostate problems, malaria, and respiratory infections (Kipkore et al, 2014; Ochwang'et al, 2014). The methanol extract of *P. africana* showed promising activity against gram negative, gram-positive bacteria and the dermatophytes according to the study done by Bii et al. (2010). According to the findings of yet another investigation, which was carried out in Kenya by Mwitari et al. (2013), extracts of Prunus africana show both antibacterial and antifungal properties. While *Euclea divinorum*, *Carissa edulis* and Prunus *africana* has a long history of traditional use in Kenya, more research is needed to determine its safety and efficacy for these medicinal purposes. Therefore, this study focused on the antimicrobial capabilities of *Euclea divinorum* Hern (Ebenaceae), *Carissa edulis*, and *Prunus africana* against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* bacteria to complement the work of other researchers.

METHODOLOGY

Plants Sample Collection

Leaves, stem barks and root of *Euclea divinorum* Hern (Ebenaceae), *Carissa edulis, Warbugia ugandensis and Prunus Africana* plants were purposively collected from Elgeyo Marakwet County in 2022. The specimen was then taken to the Department of Botany Herbarium for authentication and assignment of a voucher specimen number.

Preparation of Plants Extracts

The collected plants' roots, leaves and stem barks were cleaned with tap water, distilled water, and then shade-dried for two weeks. They were processed into fine powders in a laboratory grinding mill. They were then placed in airtight bags, labelled, and stored in the dark until extraction. Approximately 50 g of each plant powder was weighed and extracted with 0.5 litres of ethanol by stirring constantly with a magnetic stirrer for three hours. The extract was filtered through filter paper and evaporated to dryness under a vacuum. The extract was then kept at 2-8 °C until it was utilised.

Culturing of Test Microorganisms

Staphylococcus aureus, *Escherichia coli*, and *Candida albicans* was employed as test cultures. The test strains were chosen for their opportunistic pathogenicity and resistance to conventional treatments. Agar diffusion were used to test antimicrobial activity (Rojas et al, 2006). In order to achieve functioning cultures, the test microorganisms were subcultured for a period of 18 hours in their nutrient medium. Each plant extract was dissolved in methanol, hexane and acetone, sterilized by filtration using a sintered glass filter, and stored at 4°C.The nutrient media for the test microorganisms were produced as directed, sanitized, and cooled to roughly 50°C. For each cultured microorganism, 5 ml sterilized distilled water will be used to make inoculated agar with 106 CFU/ml. Using a 100ml measuring cylinder, quickly and gently pour the inoculated nutritional medium into 90mm petri dishes, delivering 20ml of seeded agar with a 3mm uniform thickness in each petri dish. The seeded agar was allowed to cool and gel. The bacteria were injected using a sterilized stainless-steel

stirrer. Following inoculation, aseptically place all ingredient discs using sterile forecep. Incubate the plates at 37°C for 24 hours. Inhibition zone was computed the next day.

Antimicrobial Assay

Mueller-Hinton agar (Difco) was used to inoculate test strain vegetative cell suspensions in sterile normal saline. About 500 mg of the ethanolic extract of each plant was triturated with 1 ml DMSO then made up to 5 ml in distilled water to give a test solution of 100 mg/l concentration for each fraction. To each well was added 0.05ml, giving a total of 5 mg of extract per well. Aseptically impregnated sterile paper discs (6.0 mm) with 0.01mL of the solutions were put on inoculation plates. After 24 and 72 h at 37 and 35°C for bacteria and yeast, respectively, distinct zones of inhibition around the test extracts indicated activity. Antibacterial and antifungal standards were chloramphenicol (0.030 mg) and fluconazole (0.025 mg) discs. Negative controls included extraction solvents.

RESULTS AND DISCUSSION

Zones of inhibition readings (in mm) for *Staphylococcus aureus* for the test solutions of *Euclea divinorum* Hern (Ebenaceae), *Carissa edulis*, and *Prunus africana* were recorded and presented in figure 1, 2 and 3. All extracts used at a concentration of 100 mg/ml.

Antimicrobial Susceptibility Effect of the root extracts of *E. divinorum C. edulis* and *P. africana* against *S. aureus, E. coli,* and *C. albican*s

After 24 hours of introducing the root extracts to the colonies on petri dishes, the inhibitory diameters of the wells were measured to test their antibacterial activity. Figure 1 shows the roots extracts of *E. divinorum C. edulis* and *P. africana* average inhibition diameters on *S. aureus, E. coli*, and *C. albican*s.

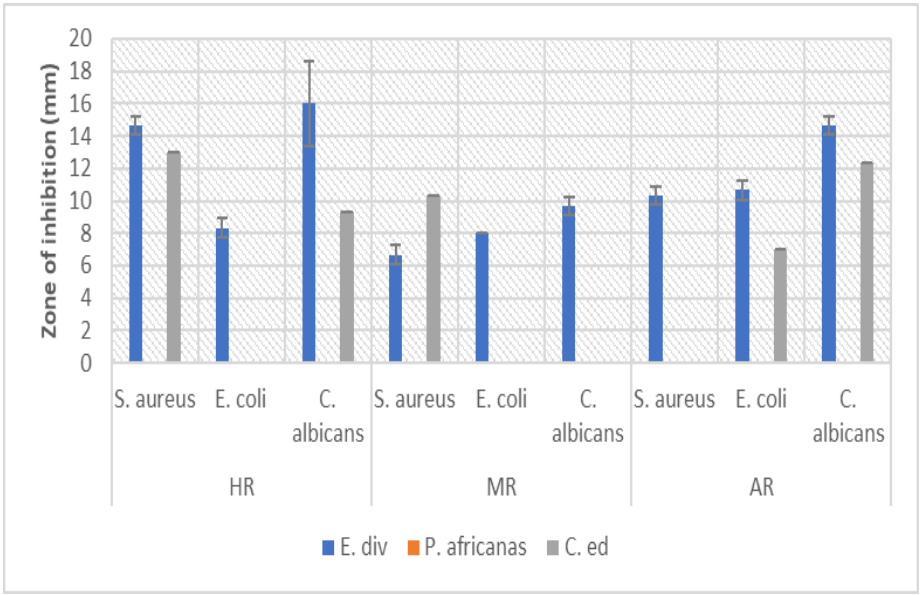

Figure 1: Zones of inhibition (mm) of the root's extracts of *E. divinorum C. edulis* and *P. africana* against *S. aureus, E. coli,* and *C. albican*s

The antimicrobial activity of root extracts of *Euclea divinorum* Hern (Ebenaceae), *Carissa edulis*, and *Prunus africana* in hexane, methanol and acetone are shown in figure 1. The results of bacterial and fungal growth inhibition showed the highest inhibition with the n-Hexane extract, followed by ethyl acetate and finally methanol. A similar study was done by Mbabazia et al. (2020) who observed that the ethanolic extract of *E. divinorum* root was the most active, with MICs of 50, 25, and 25 g/ml for *Staphylococcus aureus, Escherichia coli*,

and *Candida albican*s, respectively. In another investigation (Mothana et al., 2009), the root methanolic extract of E. divinorum exhibited antimicrobial activity against *S. Aureus* and *E. coli*, and *Candida maltosa* strains. However, a study done by Ngari et al. (2013) reported that aqueous and DCM/methanol extracts of *E. divinorum* roots had no antimicrobial activity against *E. Coli*. There was no reported antimicrobial activity of root extracts of *Prunus africana* against *Staphylococcus aureus, Escherichia coli* and *Candida albican*s though Bii et al. (2010) reported good activity of methanol extracts of *prunus africana* against *Staphylococcus aureus* bacterial and *Candida albican*s fungal strains.

The results of this study were further supported by those of Madivoli et al. (2018), who noted in their conclusion that the presence of secondary metabolites in *Prunus africana* extracts exhibited moderate antibacterial activity against the selected microorganisms. *Carissa edulis* on the other hand had antimicrobial activity against the bacterial strains and fungal strain. Also, Abdu et al. (2008) observed that the ethanol and pet-ether fractions of *Carissa edulis* roots were active against *S. aureus* and *E. coli* at high concentrations. This demonstrates that *C. edulis* contains bioactive chemicals with potential therapeutic and preventative value and bolsters the idea that it can treat bacterial and fungal illnesses. Another study reported that *C. edulis* roots were active only on *S. aureus* recording an inhibition diameter of 8.25mm.

Antimicrobial Susceptibility Effect of the leaves eextracts of *E. divinorum C. edulis* and *P. africana* against *S. aureus, E. coli*, and *C. albican*s

The sensitivity of *S. aureus, E. coli*, and *C. albican*s to the leaves extracts of *E. divinorum C. edulis* and *P. africana* were tested. The results shown (figure 2) are the average of inhibition zones for hexane, methanol and acetone extracts.

The findings presented in figure 2 as minimum inhibition concentrations (MIC) indicated that leave extracts possess varying anti microbial potencies in hexane, methanol and acetone. The crude hexane leaf extract of *E. divinorum* and *P. africana* at the concentration of (100 mg/ml), exhibited greater inhibition on *S. aureus, E. coli*, and *C. albican*s. Findings from this study are in line with the previous study conducted by Kilonzo et al., (2019) which reported that *E. divinorum* leaf ethyl acetate and E. *divinorum* leaf aqueous extracts displayed antimicrobial activity against *C. Albicans*.

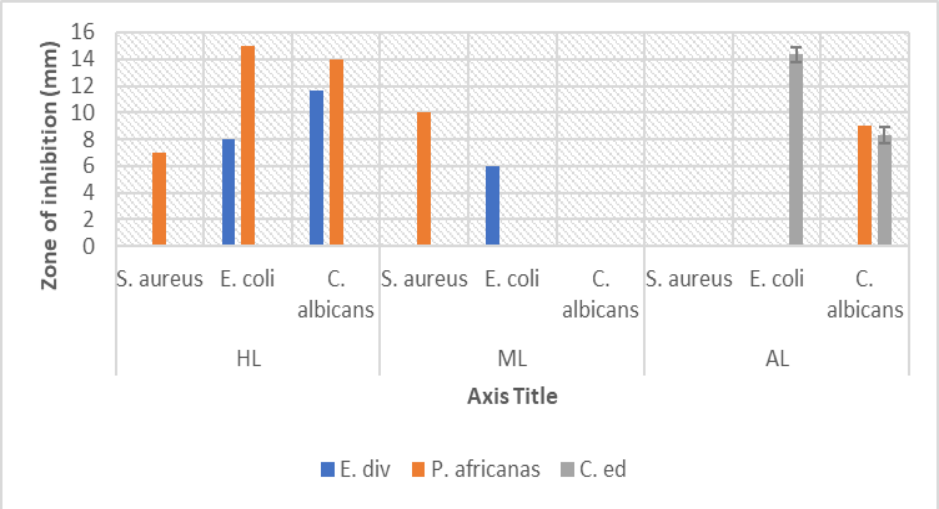

Figure 2: Zones of inhibition (mm) of the leaves extract of *E. divinorum, C. edulis* and *P. africana* against *S. aureus, E. coli*, and *C. albican*s

Antimicrobial Susceptibility Effect of the stem bark eextracts of *E. divinorum C. edulis* and *P. africana* against *S. aureus, E. coli*, and *C. albican*s

Figure 3 shows the stem bark extracts of *E. divinorum C. edulis* and *P. africana* average inhibition diameters on *S. aureus, E. coli*, and *C. albican*s.

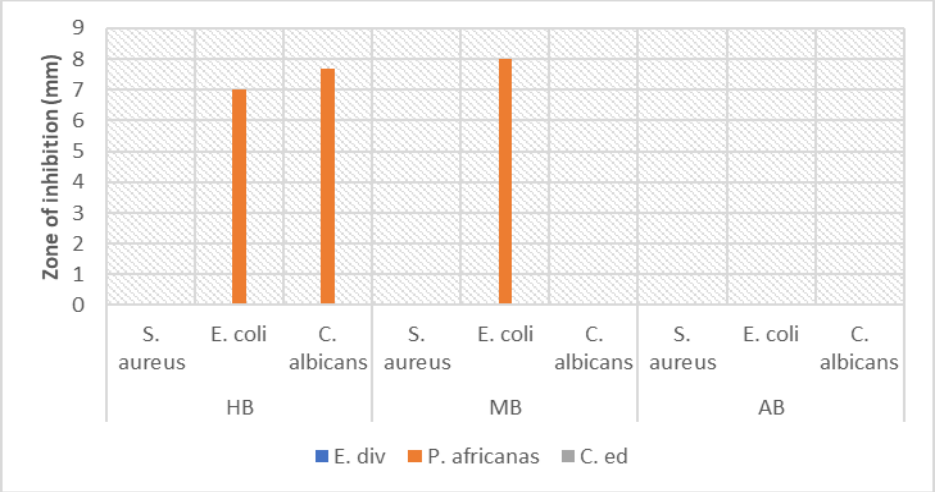

Figure 3: Zones of inhibition (mm) of the stem bark extract of *E. divinorum, C. edulis* and *P. africana* against *S. aureus, E. coli*, and *C. albican*s

From the results presented in figure 3, it is evident that the methanol stem bark extract of *P. africana* was only active against *E. coli*, and *C. albican*s. The stem bark extract of *E. divinorum* and C. *edulis* were not against *S. aureus, E. coli*, and *C. albican*s. A similar study was done by Thoithi et al. (2014) on the antimicrobial properties of some medicinal plants of the Luo community of Kenya and reported that only the roots of *C. edulis* was active against *S. aureus, E. coli*, and *C. albican*s, the stem barks were not active. It is therefore assumed that these stem bark extracts of *P. africana* are potential source of drug leads for the management of infections caused by *S. aureus and E. coli* and not the stem barks of *E. divinorum* and C. *edulis.*

CONCLUSION AND RECOMMENDATION

The roots, leaves and stem bark extracts of *E. divinorum, C. edulis* and *P. africana* against *S. aureus, E. coli*, and *C. albican*s exhibit varying degrees of antimicrobial activities against *S. aureus* and *E. coli* bacterial strain and *C. albican*s fungal strain. *E. divinorum* and *C. edulis* roots extracts exhibited antimicrobial potency against *S. aureus, E. coli*, and *C. albican*s while the leaves of *E. divinorum* and *P. africana* showed antimicrobial activity against *S. aureus* and *E. coli* bacterial strain. Lastly, the methanol stem bark extract of *P. africana* was only active against *E. coli*, and *C. albican*s however, the stem bark extract of *E. divinorum* and C. *edulis* were not against *S. aureus, E. coli*, and *C. albican*s. It is therefore recommended that root extracts of *E. divinorum* and *C. edulis* and the stem bark extracts of *P. Africana* may provide potential sources for the development of alternative antibacterial agents while *E. divinorum* and *C. edulis* agents may provide potential sources for further development of antifungal agents for the treatment of diseases.

REFERENCES

Abdu, K. B., Khan, M. E., & Rumah, M. M. (2008). Antimicrobial activity and phytochemical screening of extracts from the root bark of Carissa edulis, against human/animal pathogens. *Continental Journal of Tropical Medicine, 2*, 1.

Abdullahi, A. A. (2011). Trends and challenges of traditional medicine in Africa. *African journal of traditional, complementary and alternative medicines*, *8*(5S).

Abera, B. (2014). Medicinal plants used in traditional medicine by Oromo people, Ghimbi District, Southwest

Ethiopia. *Journal of ethnobiology and ethnomedicine*, *10*, 1-15.
Adu-Gyamfi, S., & Anderson, E. (2019). Indigenous medicine and traditional healing in Africa: a systematic synthesis of the literature. *Philosophy, Social and Human Disciplines*, *1*, 69-100.
Al-Fatimi, M., Friedrich, U., & Jenett-Siems, K. (2005). Cytotoxicity of plants used in traditional medicine in Yemen. *Fitoterapia*, *76*(3-4), 355-358.
Alvin, A., Miller, K. I., & Neilan, B. A. (2014). Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. *Microbiological research*, *169*(7-8), 483-495.
Bii, C., Korir, K. R., Rugutt, J., & Mutai, C. (2010). The potential use of Prunus africana for the control, treatment and management of common fungal and bacterial infections. *Journal of medicinal plants research*, *4*(11), 995-998.
Bodeker, G. (2005). *WHO global atlas of traditional, complementary and alternative medicine* (Vol. 1). World Health Organization.
Charles-Dominique, T., Staver, A. C., Midgley, G. F., & Bond, W. J. (2015). Functional differentiation of biomes in an African savanna/forest mosaic. *South African Journal of Botany*, *101*, 82-90.
Che, C. T., George, V., Ijinu, T. P., Pushpangadan, P., & Andrae-Marobela, K. (2017). Traditional medicine. In *Pharmacognosy* (pp. 15-30). Academic Press.
Chebii, W. K. (2016). Assessment of Kenya's Montane Forest Ecosystems: A Case Study on the Cherangani Hills in Western Kenya. *International Journal of Science Arts and Commerce*, *1*, 46-58.
Cragg, G. M., & Newman, D. J. (2001). Natural product drug discovery in the next millennium. *Pharmaceutical biology*, *39*(sup1), 8-17.
Elujoba, A. A., Odeleye, O. M., & Ogunyemi, C. M. (2005). Traditional medicine development for medical and dental primary health care delivery system in Africa. *African journal of traditional, complementary and alternative medicines*, *2*(1), 46-61.
Ezeome, E. R., & Anarado, A. N. (2007). Use of complementary and alternative medicine by cancer patients at the University of Nigeria Teaching Hospital, Enugu, Nigeria. *BMC complementary and alternative medicine*, *7*(1), 1-8.
Gladys, C. (2020). *Abundance And Conservation Status of Prunus Africana in Western Mau Forest, Kenya* (Doctoral Dissertation, School Of Pure And Applied Sciences, Kenyatta University).
Gyasi, R. M., Asante, F., Abass, K., Yeboah, J. Y., Adu-Gyamfi, S., & Amoah, P. A. (2016). Do health beliefs explain traditional medical therapies utilisation? Evidence from Ghana. *Cogent Social Sciences*, *2*(1), 1209995.
Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine*, *6*(09), 635-642.
Ibrahim, H., Oyi, R. A., Ehinmidu, J. O., Musa, K. Y., & Bright, N. T. (2010). Antimicrobial activity of the water extracts of the leaves and fruits of Carissa edulis Vahl (Apocynaceae). *Journal of medicinal plants research*, *4*(11), 1028-1032.
Ingram, V. J., van Loo, J., Vinceti, B., Dawson, I., Muchugi, A., Duminil, J., ... & Sunderland, T. (2015). *Ensuring the future of the pygeum tree (Prunus africana). Factsheet*. LEI Wageningen UR.
Jachak, S. M., & Saklani, A. (2007). Challenges and opportunities in drug discovery from plants. *Current science*, 1251-1257.
Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1), 1-7.
Kaingu, C. K., Oduma, J. A., & Kanui, T. (2012). Preliminary investigation of contractile activity of Ricinus communis and Euclea divinorum extracts on isolated rabbit uterine strips. *Journal of ethnopharmacology*, *142*(2), 496-502.
Kilonzo, M., Rubanza, C., Richard, U., & Sangiwa, G. (2019). Antimicrobial activities and phytochemical analysis of extracts from Ormocarpum trichocarpum (Taub.) and Euclea divinorum (Hiern) used as traditional medicine in Tanzania. *Tanzania Journal of Health Research*, *21*(2), 1-12.
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.
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.
Kloos, H., Mariam, D. H., Kaba, M., & Tadele, G. (2013). Traditional medicine and HIV/AIDS in Ethiopia: Herbal medicine and faith healing: A review. *Ethiopian Journal of Health Development*, *27*(2), 141-155.
Komakech, R., & Kang, Y. (2019). Ethnopharmacological potential of African cherry [Prunus africana]. *Journal of Herbal Medicine*, *17*, 100283.
Komakech, R., Yang, S., Song, J. H., Choi, G., Kim, Y. G., Okello, D., ... & Kang, Y. (2022). Assessment of anatomical characteristics of the medicinal plant African cherry (Prunus africana) for its accurate taxonomic identification. *Journal of Plant Biotechnology*, *49*(2), 139-144.
Madani, I. K. R. A. M., Ali, L. I., & Nur, E. E. (2017). Evidences from morphological investigations supporting APGIII and APGIV classification of the family Apocynaceae Juss., nom. cons. *European Academic Journal of Biological Diversity*, *11*(4), 187-193.
Madivoli, E. S., Maina, E. G., Kairigo, P. K., Murigi, M. K., Ogilo, J. K., Nyangau, J. O., ... & Kipyegon, C. (2018). In vitro antioxidant and antimicrobial activity of Prunus africana (Hook. f.) Kalkman (bark extracts) and Harrisonia abyssinica Oliv. extracts (bark extracts): A comparative study. *Journal of Medicinal Plants for Economic Development*, *2*(1), 1-9.
Mahomoodally, M. F. (2013). Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-based complementary and alternative medicine*, *2013*.

Mahwasane, S. T., Middleton, L., & Boaduo, N. (2013). An ethnobotanical survey of indigenous knowledge on medicinal plants used by the traditional healers of the Lwamondo area, Limpopo province, South Africa. *South African Journal of Botany*, *88*, 69-75.

Mbabazia, I., Wangila, P., & K'Owinoc, I. O. (2020). Comparison of the phytochemical composition of Euclea divinorum Hiern (Ebenaceae) leaves, tender stems and root bark. *Adv J Chem B*, *3*(3), 218-42.

Mothana, R. A., Lindequist, U., Gruenert, R., & Bednarski, P. J. (2009). Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. *BMC complementary and alternative medicine*, *9*, 1-11.

Mothana, R. A., Lindequist, U., Gruenert, R., & Bednarski, P. J. (2009). Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqotra. *BMC complementary and alternative medicine*, *9*, 1-11.

Mwitari, P. G., Ayeka, P. A., Ondicho, J., Matu, E. N., & Bii, C. C. (2013). Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus. *PloS one*, *8*(6), e65619.

Ngari, F. W., Gikonyo, N. K., Wanjau, R. N., & Njagi, E. M. (2013). Safety and antimicrobial properties of Euclea divinorum Hiern, chewing sticks used for management of oral health in Nairobi County, Kenya. *J Pharm Biomed Sci*, *3*(3), 1-8.

Ngiti, J. G. (2019). *Factors Associated with Herbal Medicine Use in Children 0-12 Years Old: A Case of Bonchari Sub County, Kisii County, Kenya* (Doctoral dissertation).

Nyamai, D. W., Mawia, A. M., Wambua, F. K., Njoroge, A., Matheri, F., Lagat, R., & Burugu, M. (2015). Phytochemical profile of Prunus africana stem bark from Kenya. *Journal of Pharmacognosy and Natural Products*, *1*(1), 8.

Nyambe, M. M. (2014). *Phytochemical and antibacterial analysis of indigenous chewing sticks, Diospyros lycioides and Euclea divinorum* (Doctoral dissertation).

Nyambe, M. M., Kwembeya, E. G., Lisao, K., & Hans, R. (2021). Oral hygiene in Namibia: A case of chewing sticks. *Journal of Ethnopharmacology*, *277*, 114203.

Ochwang'i, D. O., Kimwele, C. N., Oduma, J. A., Gathumbi, P. K., Mbaria, J. M., & Kiama, S. G. (2014). Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. *Journal of Ethnopharmacology*, *151*(3), 1040-1055.

Ogoma, B. O., Omondi, S. F., Ngaira, J., & Kimani, J. W. (2021). Molecular diversity of arbuscular mycorrhizal fungi (AMF) associated with Carissa edulis, an Endangered Plant Species along Lake Victoria Basin of Kenya. *International Journal of Forestry Research*, *2021*, 1-10.

Oguntibeju, O. O. (2018). Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. *Journal of inflammation research*, 307-317.

Okello, S. V., Nyunja, R. O., Netondo, G. W., & Onyango, J. C. (2010). Ethnobotanical study of medicinal plants used by Sabaots of Mt. Elgon Kenya. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*(1).

Okoronkwo, I., Onyia-Pat, J. L., Okpala, P., Agbo, M. A., & Ndu, A. (2014). Patterns of complementary and alternative medicine use, perceived benefits, and adverse effects among adult users in Enugu Urban, Southeast Nigeria. *Evidence-Based Complementary and Alternative Medicine*, *2014*.

Omara, T., Sadia, B. O., Mbabazi, I., & Okwir, A. (2022). Ethnobotany, Phytochemistry, Ethnopharmacology, and toxicity of Euclea divinorum Hern (Ebenaceae): a review. *Journal of the Turkish Chemical Society Section A: Chemistry*, *9*(2), 355-372.

Opande, G. T. (2022). Phytochemical Screening and Antimicrobial Properties of Carissa edulis Extracts Obtained from Kaimosi Forest, Vihiga County, Kenya. *European Journal of Medicinal Plants*, *33*(10), 11-18.

Ozioma, E. O. J., & Chinwe, O. A. N. (2019). Herbal medicines in African traditional medicine. *Herbal medicine*, *10*, 191-214.

Pan, S. Y., Litscher, G., Gao, S. H., Zhou, S. F., Yu, Z. L., Chen, H. Q., ... & Ko, K. M. (2014). Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. *Evidence-based complementary and alternative medicine*, *2014*.

Payyappallimana, U. (2010). Role of traditional medicine in primary health care. *Yokohama Journal of Social Sciences*, *14*(6), 57-75.

Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, *6*(11), 1.

Rahmatullah, M., Mukti, I. J., Haque, A. K. M. F., Mollik, M. A. H., Parvin, K., Jahan, R., ... & Rahman, T. (2009). An ethnobotanical survey and pharmacological evaluation of medicinal plants used by the Garo tribal community living in Netrakona district, Bangladesh. *Advances in Natural and Applied Sciences*, *3*(3), 402-418.

Rastogi, S., Pandey, M. M., & Rawat, A. K. S. (2016). Traditional herbs: a remedy for cardiovascular disorders. *Phytomedicine*, *23*(11), 1082-1089.

Rubegeta, E., Makolo, F., Kamatou, G., Enslin, G., Chaudhary, S., Sandasi, M., ... & Viljoen, A. (2022). The African cherry: A review of the botany, traditional uses, phytochemistry, and biological activities of Prunus africana (Hook. f.) Kalkman. *Journal of Ethnopharmacology*, 116004.

Singh, R. (2015). Medicinal plants: A review. *Journal of plant sciences*, *3*(1-1), 50-55.

Somvanshi, R. (2006). Veterinary medicine and animal keeping in ancient India. *Asian Agri-History*, *10*(2), 133-146.

Thoithi, G. N., Ndwigah, S. N., & Maima, A. O. (2014). Antimicrobial properties of some medicinal plants of the Luo Community of Kenya.

Trotter, R. T., & Logan, M. H. (2019). Informant consensus: a new approach for identifying potentially effective medicinal plants. In *Plants in indigenous medicine & diet* (pp. 91-112). Routledge.

Ukwubile, C. A., Malgwi, T. S., Angyu, A. E., Otalu, O., & Bingari, M. S. (2020). Review of antiviral medicinal plants used in Taraba state Nigeria: A possible source for Covid-19 drug discovery. *Journal of Scientific Research in Medical and Biological Sciences*, *1*(2), 1-23.

Wadud, A., Prasad, P. V., Rao, M. M., & Narayana, A. (2007). Evolution of drug: a historical perspective. *Bull Indian Inst Hist Med Hyderabad*, *37*(1), 69-80.

World Health Organization. (1978). *The promotion and development of traditional medicine: report of a WHO meeting [held in Geneva from 28 November to 2 December 1977]*. World Health Organization.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559.

Zhang, A., Sun, H., Qiu, S., & Wang, X. (2013). Advancing drug discovery and development from active constituents of yinchenhao tang, a famous traditional chinese medicine formula. *Evidence-Based Complementary and Alternative Medicine*, *2013*.