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Original article

Antimicrobial activities and phytochemical analysis of *Harrisonia abyssinica* (Oliv) and *Vepris simplicifolia* (Verd) extracts used as traditional medicine in Tanzania

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ABSTRACT

The present study aimed to determine *in vitro* antimicrobial activities and phytochemical analysis of *Harrisonia abyssinica* and *Vepris simplifolia* which are commonly used as traditional medicine in Tanzania. Sensitivity of bacterial and fungal species against plants extracts were determined using serial microdilution method. In this method, the lowest inhibitory concentration which prevented microbial growth considered as minimum inhibitory concentration (MIC). The study also evaluated phytochemical compounds present in the leaf, stem and root barks of *H. abyssinica*. It was revealed that eight extracts from *H. abyssinica* inhibited growth of three bacteria namely *Staphylococcus aureus*, *Enterococcus faecium* and *Streptococcus uberis* at MIC value less than 1 mg/mL. It was further revealed that, three extracts from *V. simplifolia* exhibited high antibacterial and antifungal activity. The preliminary phytochemical analysis revealed presence of various phytochemicals namely alkaloids, terpenoid, flavonoid, tannin and saponin. It was concluded that presence of large number of phytochemicals in the plant extracts may be associated with pharmacological properties of *H. abyssinica* and *V. simplifolia* and therefore this study provide alternative to synthetic antimicrobial agents.

1. Introduction

The essentiality of medicinal plants remains to enlarge crosswise the world with various individuals resourcing to these products for treatment of many disorders such as asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems, hemorrhagic diarrhea, stomach and cardiovascular disease (WHO, 2014). According to WHO (2007) about 80% of the world's population depends on medicinal plants for their health. Herbal plants therefore have been of an ample value in worldwide for many centuries (Pokharen et al., 2011). Globally, about 72,000–77,000 plant species are being utilized for medicinal purposes (Rao and Rajput, 2010). In Tanzania, about 12,000 plant species have been documented and about 10% are likely to be used by humans for their health care (Mahunnah et al., 2012). Some of these reported plants include *Harrisonia abyssinica* (Oliv) and *Vepris simplicifolia* (Verd).

The *H. abyssinica* which belong to family Rutaceae is a thorny, evergreen shrub that branches from base and wide spreading. The plant usually grows between 6 and 13 m tall (Burrows et al. 2018). The plant is used as herbal medicine in many countries of Africa. Leaves and roots in the form of powder are used to cure complaints such as venereal diseases, malaria, diarrhoea, haemorrhoids, diabetes, urinary problems, general body pain, snakebites and intestinal worms (Da Silva et al., 2004). In Africa, *H. abyssinica* is commonly found in Tanzania, Sierra Leone, Cameroon, Sudan, Ethiopia, Uganda, Kenya, Angola, Zambia and Mozambique (Da Silva et al. 2004).

Regarding *V. simplicifolia* (Rutaceae) is an evergreen shrub or tree, usually growing up to10 metres tall, with exception to some species which reach 20 m high. In African continent, this specie is originated in Ethiopia, Kenya, Tanzania and Uganda where it is mostly found in dry forest, riverine thicket or woodland, evergreen rocky bushland and montane thicket (Lovett et al., 1990). Traditionally, the leaf powder of a plant is taken in the treatment of

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pneumonia, pleurisy, leprosy, stomach-ache, backache, gonorrhea and brucellosis (Ombito et al. 2020). The fruits are chewed to relieve toothache while root decoction is used to treat stomach-ache, backache, leprosy, gonorrhoea and brucellosis (Kokwaro, 1993).

Taking into consideration the contribution of *H. abyssinica* and *V. simplicifolia* in the management of various ailments, it is justifiable that the plants are among the herbal species which are recognized for various remedial applications. Despite of their medicinal value, less is known regarding the medicinal potential and secondary metabolites which are responsible for healing effects of such plants. Current study therefore aimed to assess the antimicrobial activities and phytochemical analysis of *H. abyssinica* and *V. simplicifolia* commonly used as traditional medicine in Tanzania.

2. Materials and methods

2.1. Materials

Petroleum ether (99.7%) purchased from Avantor Perfomance Ltd, India. Dimethyl sulphoxide (DMSO) (99.9%) and ethyl acetate (85.5%) purchased from RFCL Ltd, Haryana, India. Nutrient broth and Nutrient agar were supplied by HIMEDIA Laboratories Pvt. Ltd, India. Five bacterial and two fungal strains namely *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecium* (ATCC 51559), *Streptococcus uberis* (ATCC BBA-854), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (Clinical isolate) respectively were obtained from the department of Parasitology and Entomology, University of Dodoma, Tanzania. Gentamycin and iodonitrotetrazolium chloride were provided by Mediatech Incorporation, USA.

2.2. Extraction process

Studied plants namely of *H. abyssinica* and *V. simplicifolia* were collected from Esilalei and Imbibya villages in Monduli and Arumeru districts respectively. The voucher specimens coded HA-001 for *H. abyssinica* and VS-002 for *V. simplicifolia* are kept at the Department of Biology Laboratory, University of Dodoma. Collected plants were air dried and crushed. For non-polar and medium polar extraction, crushed samples of 250 g were macerated in petroleum ether and ethyl acetate. The extracts were filtered using Whatman filter paper and solvents were evaporated using rotary evaporator. For polar extraction, the same crushed materials were added to a 1 L of distilled water at 70 °C and allowed to cool at 35 °C in a water bath. The extracts were sieved and centrifuged for about 15 min. The supernatant was collected and filtered using filter paper and allowed to freeze in a closed flask in the freezer to eliminate water by sublimation. To ensure complete extraction, each solution was shaken after every 40 min for five hours and allowed to settle for 48 h as described by Olowa and Nuñeza (2013). Extract yields (% yield = W2/W1*100, whereby, W1 is the weight of pulverized sample before extraction, while W2 is the weight of pulverized sample after extraction) of between 0.12 and 4.52% were obtained.

2.3. Determination of antimicrobial activities

In this study, minimum inhibitory concentrations (MIC) were determined using serial microdilution method as described by Ellof (1998). Plates were loaded with 50 µL of nutrient broth and Sabouraud's dextrose media in each well followed by about 50 µL of 100 mg/mL extract into the first row to make volume of 100 µL. About 50 µL of the solution were drawn from first row and put into the next row. This process repeated down the columns to the last wells and the last 50 µL were discarded. Thereafter, about 50 µL of 0.5 McFarland standard turbidity microbes were added to each well. Gentamicin and fluconazole were used in two rows of each plate to serve as positive controls while nutrient broth and saboraud's dextrose served as negative controls and DMSO as solvent controls. Plates were incubated at 37 °C for 24 h. Minimum inhibition concentrations for each extract were determined by adding 10 µL of iodonitrotetrazolium chloride followed by incubation at 32 °C for 1 h. Sensitivity of pathogen was indicated by change of colour to pink. Lowest concentration that showed no pathogen growth was regarded as MIC.

2.4. Phytochemical screening

Preliminary phytochemical screening of the *H. abyssinica* leaf aqueous (HALA), *H. abyssinica* stem bark ethyl acetate (HASEA), *H. abyssinica* stem bark aqueous (HASA) and *H. abyssinica* root bark ethyl acetate (HAREA) extracts were tested for alkaloid, terpenoid, flavonoid, tannin and saponin. The choice of these extracts based on their highest potency against antimicrobial activity. The results are indicated as (+, ++, +++) for the presence and (−) for the absence of phytochemicals.

2.5. Test for alkaloid

Few drops of Dragendroff's were applied to a test tube of about 1 ml of extract and colour change was detected. Occurrence of an orange colour was a sign of the presence of alkaloids (Firdouse and Alam, 2011).

2.6. Test for terpenoid

About 5 ml of plant extract was applied to a 3 ml of chloroform and 2 ml of concentrated sulphuric acid (H_2SO_4). The presence of terpenoids was observed by reddish brown colour (Edeoga et al., 2005).

2.7. Test for flavonoids

In this test, 2 ml of plant extract was treated with 5 drops of dilute sodium hydroxide (NaOH), followed by diluted hydrochloric acid (HCl). A yellow solution with NaOH turned colorless with dilute HCl indicated existence of flavonoids (Onwukaeme et al., 2007).

2.8. Test for tannin

About 2 ml of tested plant extract was stirred with 3 ml of distilled water and five drops of ferric chloride ($FeCl_3$) were added. Formation of dark blue precipitate was the indication of tannins (Kumar et al., 2007).

2.9. Test for saponin

In this test, about 5 ml of tested extract was shaken with 5 ml of distilled water in a test tube. The formation of foam was considered as an indication of the presence of saponins (Parekh and Chanda, 2007).

3. Results

The antimicrobial activities of *H. abyssinica* and *V. simplicifolia* extracts were evaluated against five bacterial and two fungal species. These species included *S. aureus*, *E. faecium*, *S. uberis*, *E. coli*, *K.*

pneumonia, *C. albicans* and *C. neoformans* respectively and results are summarized in Tables 1 and 2. These findings are presented as minimum inhibition concentrations which indicate that plant extracts possessed antimicrobial properties. Findings from this study observed that out of nine extracts from *H. abyssinica*, eight extracts inhibited growth of three bacteria namely *S. aureus*, *E. faecium* and *S. uberis* at MIC value less than 1 mg/mL.

The *H. abyssinica* root bark ethyl acetate (HAREA) extract, root bark petroleum ether (HARPE) extract and root bark aqueous (HARA) extract displayed antimicrobial activity against *S. aureus*, *E. faecium* and *S. uberis* with narrow MIC range of 0.011–0.047 mg/mL. Additionally, *H. abyssinica* root bark ethyl acetate (HAREA) extract showed high MIC value of 0.188 mg/mL against *C. albicans* and *C. neoformans*. The *H. abyssinica* stem bark ethyl acetate (HASEA) extract had antimicrobial properties with MIC range of 0.011–0.75 mg/mL against five out of seven microbes tested followed by *H. abyssinica* stem bark petroleum ether (HASPE) and leaf ethyl acetate (HALEA) extracts with MIC range between 0.188 and 0.75 mg/mL. These microbes include *S. aureus, E. faecium, S. uberis, C. albicans* and *C. neuformas*. The *H. abyssinica* leaf aqueous (HALA) extract had high antimicrobial potency against *S. aureus, S. uberis* and *E. faecium* which is evidenced by MIC values of 0.011 mg/mL and 0.75 mg/mL respectively.

Regarding the *V. simplicifolia,* only three out of nine plant extracts inhibited growth of pathogenic microbes with MIC values of less than 1 mg/mL. However, the uppermost antimicrobial activity was shown by *V. simplicifolia* leaf ethyl acetate (VSLEA), *V. simplicifolia* leaf aqueous (VSLA) and *V. simplicifolia* root bark (VSRA) aqueous extracts against tested fungal species and one bacteria namely *C. albicans, C. neuformas* and *S. uberis* respectively at MIC value of 0.75 mg/mL.

The phytochemical screening of *H. abyssinica* leaf aqueous (HALA) extract, *H. abyssinica* stem barks ethyl acetate (HASEA) extract, *H. abyssinica* stem barks aqueous (HASA) extract and *H. abyssinica* root barks ethyl acetate (HAREA) revealed presence of main categories of phytochemical compounds which are alkaloids, terpenoid, flavonoid, tannin and saponin as shown in Table 3.

4. Discussion

Justification of ethnomedical information of plants used as herbal medicines has been a strategy in the discovery of bioactive phytochemicals (Bonjar, 2004). In spite of this, some plant species have not been screened for their biological capabilities. That is why this study is reporting novelty of antimicrobial activity of *H. abyssinica* and *V. simplicifolia* which are found in Tanzania. According to Rios and Recio (2005) an extract with less than 1 mg/mL should be taken for consideration. In this study, eleven out of eighteen extracts tested displayed antimicrobial activity with MIC of less than 1 mg/mL suggesting that such plant extracts are regarded as potential drug sources.

In this regard, *H. abyssinica* root bark ethyl acetate (HAREA) extract, root bark petroleum ether (HARPE) extract and root bark aqueous (HARA) extract which demonstrated antimicrobial potency against *S. aureus*, *E. faecium* and *S. uberis* with MIC range of 0.011–0.047 mg/mL are possible sources of herbal drugs to be used to cure diseases caused by these pathogenic microbes. These results validate the earlier reported uses of *H. abyssinica* root bark in Tanzania and Kenya for the management of fever, venereal diseases, plague and tuberculosis (Schmelzer et al., 2010; Kokwaro, 1993; Beentje, 1994). The current study results are also in line with results of Cyrus et al. (2008) who observed significant antibacterial activity of methanol root bark extracts of the similar plant species against *P. aeruginosa*, *E.* coli, *B. cereus* and *S. aureus*. Another study carried out by Madivoli et al. (2018) on antimicrobial activity of *H. abyssinica* root bark extract by disc diffusion method in Kenya reported that the extract was active against *S. aureus, B. subtilis, P. aeruginosa, E. coli* and *C. albicans* with an average zone of inhibition (ZOI) of 7–10 mm.

Likewise, *H. abyssinica* stem bark ethyl acetate (HASEA) and *H. abyssinica* stem bark petroleum ether (HASPE) showed potential sources of drug leads for the management of *S. aureus, E. faecium, S. uberis, C. albicans* and *C. neuformas* as evidenced by MIC range of 0.011–0.75 mg/mL (Table 1). In this study, it was further observed that *H. abyssinica* stem bark aqueous (HASA) extract inhibited the growth of *S. aureus, E. faecium* and *S. uberis* with MIC values of 0.094 and 0.011 mg/mL respectively. The high susceptibility shown by *S. aureus, E. faecium, S. uberis, C. albicans* and *C. neuformas* towards stem bark extracts of *H. abyssinica* implying that the stem bark might comprise phytochemicals that can be considered in the treatment of infections caused by these pathogenic microbes. Studies on phytochemicals have revealed that plants which are rich in a wide range of phytochemicals such as tannins, terpenoids, alkaloids and flavonoids which reported also in this study (Table 3) have been found to possess antimicrobial potency (Cowan, 1999). Results from this study are similar with a study conducted by Nthiga et al. (2016) on phytochemical investigations of medicinal plants growing in Kenya who observed presence of alkaloids, flavonoids, steroids, saponins, phenolics and terpernoids in the *H. abyssinica* stem bark.

It was observed that *H. abyssinica* leaf aqueous (HALA) and leaf ethyl acetate (HALEA) extracts had high activities against *S. aureus, E. faecium, S. uberis*, *C. albicans* and *C. neuformas* respectively which warrant discovery of drugs for the treatment of diseases associated with these pathogenic microbes. This is evidenced from the antimi-

Table 1
Antimicrobial activity of leaf, stem and root bark extracts from *H. abyssinica.*

Plant Extract	% Yield	Minimum Inhibitory Concentrations in mg/ml						
		S. aureus	*E. faecium*	*S. uberis*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. neuformas*
HALPE	0.12	6	>6	>6	>6	>6	>3	>3
HALEA	0.22	0.188	0.75	0.188	>6	>6	0.375	0.75
HALA	2.45	0.011	0.75	0.011	>6	>6	1.5	1.5
HASPE	3.12	0.094	0.047	0.047	>6	>6	0.375	0.75
HASEA	0.63	0.094	0.023	0.011	>6	>6	0.375	0.75
HASA	3.81	0.094	0.094	0.011	>6	>6	3	3
HARPE	4.01	0.047	0.047	0.023	>6	>6	0.75	0.75
HAREA	3.25	0.023	0.023	0.011	>6	>6	0.188	0.188
HARA	4.32	0.047	0.047	0.023	>6	>6	3	3
Gentamycin		0.0625	0.0625	0.0625	0.0625	0.0625	N/A	N/A
Fluconazole		N/A	N/A	N/A	N/A	N/A	1.56	0.78

Key: HALPE = *H. abyssinica* leaf petroleum ether extract, HALEA = *H. abyssinica* leaf ethyl acetate extract, HALA = *H. abyssinica* leaf aqueous extract, HASPE = *H. abyssinica* stem bark petroleum ether extract, HASEA = *H. abyssinica* stem bark ethyl acetate extract, HASA = *H. abyssinica* stem bark aqueous extract, HARPE = *H. abyssinica* root bark petroleum ether extract, HAREA = *H. abyssinica* root bark ethyl acetate extract, HARA = *H. abyssinica* root bark aqueous extract.

Table 2
Antimicrobial activity of leaf, stem and root bark extracts from *V. simplicifolia.*

Plant Extract	% Yield	Minimum Inhibitory Concentrations in mg/ml						
		S. aureus	*E. faecium*	*S. uberis*	*E. coli*	*K. pneumoniae*	*C. albicans*	*C. neuformas*
VSLPE	1.12	>6	>6	>6	>6	>6	>3	>3
VSLEA	0.32	>6	3	>6	>6	>6	0.75	0.75
VSLA	3.25	>6	>6	>6	>6	>6	0.75	0.75
VSSPE	4.11	>6	>6	>6	>6	>6	>3	>3
VSSEA	0.33	>6	6	6	>6	>6	>3	>3
VSSA	2.71	>6	6	6	>6	>6	>3	>3
VSRPE	0.12	3	>6	>6	>6	>6	3	3
VSREA	4.52	1.5	1.5	>6	>6	>6	3	3
VSRA	4.12	3	3	0.75	>6	>6	3	3
Gentamycin		0.0625	0.0625	0.0625	0.0625	0.0625	N/A	N/A
Fluconazole		N/A	N/A	N/A	N/A	N/A	1.56	0.78

Key: VSLPE = *V. simplicifolia* leaf petroleum ether extract, VSLEA = *V. simplicifolia* leaf ethyl acetate extract, VSLA = *V. simplicifolia* leaf aqueous extract, VSSPE = *V. simplicifolia* stem bark petroleum ether extract, VSSEA = *V. simplicifolia* stem bark ethyl acetate extract, VSSA = *V. simplicifolia* stem bark aqueous extract, VSRPE = *V. simplicifolia* root bark petroleum ether extract, VSREA = *V. simplicifolia* root bark ethyl acetate extract, VSRA = *V. simplicifolia* root bark aqueous extract.

Table 3
Phytochemical screening of HALA, HASEA, HASA and HAREA extracts.

Phytochemical Components	Plant Extracts			
	HALA	HASEA	HASA	HAREA
Alkaloid	+ +	+ +	+ + +	+ + +
Terpenoid	+ + +	+	+	+ +
Flavonoid	+ +	+ + +	+ +	+
Tannin	+	+	+	–
Saponin	–	+ +	–	+

Key: + = mild positive; + + = positive; + + + = highly positive; – = negative; HALA = *H. abyssinica* leaf aqueous extract; HASEA = *H. abyssinica* stem barks ethyl acetate extract; HASA = *H. abyssinica* stem barks aqueous extract; HAREA = *H. abyssinica* root barks ethyl acetate.

crobial activity of *H. abyssinica* leaf aqueous (HALA) and leaf ethyl acetate (HALEA) extracts against such pathogenic microbes with MIC range of 0.011–0.75 mg/mL. These results endorse the local uses of *H. abyssinica* leaf by Akamba people in Machakosi, Kenya for treatment of pneumonia, syphilis, infertility, malaria, stomach ache and eye ointment (Cyrus et al., 2008). Additionally, a study conducted by Anani et al. (2000) on antimicrobial activities of medicinal plants reported uses of *H. abyssinica* leaf by ethnic groups in Togo for the management of diabetes and wound healing. Another study conducted by Balde et al. (1995) in Guinea observed that aerial parts of the same plant is commonly used in traditional medicine for the treatment of menstrual problems, stomach pains, gonorrhea, skin diseases, tuberculosis, bilharzia and infertility.

Regarding *V. simplicifolia,* the leaf ethyl acetate (VSLEA) and aqueous (VSLA) extracts had good antifungal potency against *C. albicans* and *C. neuformas* with MIC value of 0.75 mg/mL. It is therefore assumed that medium and polar compounds respectively are responsible for the both fungal species tested. Additionally, the *V. simplicifolia* root bark aqueous (VSRA) extract selectively inhibited growth of *S. uberis* with same MIC value (0.75 mg/mL). Findings from this study therefore suggest the possible sources of antimicrobial drugs and therefore feasible tool for improve drug novelty. The present study findings are also in line with the results of Mutie et al. (2020) who reported that leaf and root extracts of *V. simplicifolia* were responsible for general body pains, malaria and pleurisy by local people in Kenya.

5. Conclusion

The extracts of *H. abyssinica* and *V. simplicifolia* exhibit varying degrees of antimicrobial activities against seven pathogenic microbes namely *S. aureus*, *E. faecium*, *S. uberis*, *E. coli*, *K. pneumonia*, *C. albicans* and *C. neoformans*. Majority of plant extracts possessed antimicrobial potency and therefore the current study supports sustainable utilization of herbal medicine. Additionally, the presence of large number of phytochemicals in these two botanicals, justifies its use for the management of various diseases. However, isolation of the active phytochemicals and develop antimicrobial drugs from these plants remained unveiled and therefore needs further investigations.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anani, K., de Souza, C., Akpagana, K., Tower, G.H.N., Arnason, J.T., Gbeassor, M., Hudson, J.B., 2000. Investigation of medicinal plants of Togo for antiviral and antimicrobial activities. J. Pharma Biol. 38 (1), 40–45.
Balde, A.M., Pieters, L., De Bruyne, T., Geerts, S., Vanden Berghe, D., Vlietinck, A., 1995. Biological investigations on *Harrisonia abyssinica*. J. Phytom. 1 (4), 299–302. https://doi.org/10.1016/S0944-7113 (11)80006-1.
Beentje, H.J., 1994. Kenya Trees; Shrubs and Lianas. National Museums of Kenya, Nairobi.
Bonjar, S., 2004. Evaluation of antibacterial properties of some medicinal plants used in Iran. J. Ethnopharmacol. 94, 301–305.
Burrows, J.E., Burrows, S.M., Lötter, M.C., Schmidt, E., 2018. Trees and shrubs of Mozambique Publishing Print Matters (Pty), Cape Town. 364p.
Cowan, M.M., 1999. Plant products as antimicrobial agents. J. Clin. Microbiol. Rev. 12 (4), 564–582.
Cyrus, W.G., Daniel, G.W., Nanyingi, M.O., Njonge, F.K., Mbaria, J.M., 2008. Antibacterial and cytotoxic activity of Kenyan medicinal plants. J. Memórias do Instituto Oswaldo Cruz. 103 (7), 650–652.
Da Silva, M.C., Izidine, S., Amude, A.B., 2004. A preliminary checklist of the vascular plants of Mozambique. Southern African Botanical Diversity Network Report No. 30 Sabonet, Pretoria. 110p.
Edeoga, H.O., Okwu, D.E., Mbaebie, B.O., 2005. Phytochemical constituents of some Nigerian medicinal plants. African J. Biotech. 4 (7), 685–688.
Ellof, J.N., 1998. A quick microplate method to determine the minimum inhibitory sensitive and concentration of plant extracts for bacteria. J. Planta Medic. 64, 711–713.
Firdouse, S., Alam, P., 2011. Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. Intern J. Phytom 3 (1), 32–35.
Kokwaro, J.O., 1993. Medicinal plants of East Africa. Published and printed by Kenya Literature Bureau, Nairobi Kenya. 176p.

Kumar, G.S., Jayaveera, K.N., Kumar, C.K., Sanjay, U.P., Swamy, B.M., Kumar, D.V., 2007. Antimicrobial effects of Indian medicinal plants against acne-inducing bacteria. Tropical J. Pharm. Res. 6 (2), 717–723.

Lovett, J.C., Ruffo, C.K., Gereau, R.E., 1990. Field Guide to the moist forest trees of Tanzania.

Madivoli, E.S., Maina, E.G., Kairigo, P.K., Murigi, M.K., Ogilo, J.K., Nyangau, J.O., Kimani, P.K., 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. J. Medicinal Plants Econ. Dev. 2 (1), 1–9.

Mahunnah, R.L., Uiso, F.C., Kayombo, E.J., 2012. Documentation of traditional medicine in Tanzania. A traditioanal medicine resource book. Dar es Salaam University Press, Dar es Salaam, Tanzania.

Mutie, F.M., Gao, L.-L., Kathambi, V., Rono, P.C., Musili, P.M., Ngugi, G., Hu, G.-W., Wang, Q.-F., 2020. An Ethnobotanical survey of a dryland botanical garden and its environs in Kenya: the Mutomo Hill Plant Sanctuary. J. Evidence-Based Compl. Alt. Med. 2020, 1–22.

Nthiga, P.M., Kamau, J.K., Safari, V.Z., Mwonjoria, J.K., Mburu, D.N., Ngugi, M.P., 2016. Antipyretic potential of methanolic stem bark extracts of *Harrisonia abyssinica* (Oliv) and *Landolphia buchananii* (Hallier F.) Stapf In Wistar Rats. J. Appl. Pharm. 8 (3), 1–7.

Olowa, L.F., Nuñeza, O.M., 2013. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from ligan City, Philippines. Int. Res. J. Biol. Sc. 2 (11), 74–77.

Ombito, J.O., Chi, G.F., Wansi, J.D., 2020. Ethnomedicinal uses, phytochemistry, and pharmacology of the genus Vepris (Rutaceae): a review. J. Ethnopharmacol. 113622

Onwukaeme, D.N., Ikuegbvweha, T.B., Asonye, C.C., 2007. Evaluation of phytochemical constituents, antibacterial activities and effect of exudates of *Pycanthus angolensis* weld warb (myristicaceae) on corneal ulcers in rabbits. Trop. J. Pharm. Res. 6 (2), 725–730.

Parekh, J., Chanda, S.V., 2007. In *Vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turkish J. Biol. 31 (1), 53–58.

Pokharen, N., Dahal, S., Anuradha, M., 2011. Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*. J. Med. Plants Res. 5 (24), 5785–5788.

Rao, B.R., Rajput, D.K., 2010. Global scenario of medicinal plants. CM I'-H PLI Conference Proceedings.; https://www.researchgate.net/publication/270512088.

Rios, J., Recio, M., 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100, 80–84.

Schmelzer, G.H., Achigan-Dako, E.G., Bosch, C.H. (Eds.), 2010. Medicinal plants of tropical Africa. Conclusions and recommendations based on plant resources of tropical Africa foundation, Nairobi, Kenya. 121–152p.

World Health Organization (WHO), 2007. Department of technical cooperation for essential drugs and traditional medicine. Guidelines for assessing quality of traditional medicines with reference to contaminants and residues. World Health Organization, Geneva.

World Health Organization (WHO). (2014). Antimicrobial resistance. Global Report on surveillance. http://apps.who.int/iris/bitstream/10665/112642/1/9789241564748_eng.pdf.