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Evaluation of Antimicrobial Activity of *Mentha piperita, Tragia involucrata* (L) and *Urtica massaica* Used as Medicinal Plants in Tanzania

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Authors' contributions

This work was carried out in collaboration between both authors. Author BAN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MK managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate antimicrobial activity of *Mentha piperita, Tragia involucrata* (L) and *Urtica massaica* used as medicinal plants in Tanzania.
Study Design: Experimental study was carried out by using 96 wells micro dilution method.
Place and Duration of Study: This study was conducted at Dodoma University, Dodoma-Tanzania, between April and July 2017.
Methodology: Minimum inhibitory concentration of plants extracts against the tested bacterial and fungal species was determined by using 96 wells micro dilution method.
Results: Plant extracts exhibited antimicrobial activity with MIC range of 1.56 mg/mL to 12.5 mg/mL. The highest activity was shown by *M. piperita* chloroform leaf extract, *M. piperita* methanol leaf extract, *T. involucrate* chloroform leaf extract, *T. involucrate* ethyl acetate leaf extract, *U. massaica* chloroform leaf extract and *U. massaica* methanol leaf extract with MIC value of 1.56 mg/mL against *K. oxytoca, E. coli, S. typhi, C. neuforman* and *P. aeruginosa.*

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Conclusion: The extracts from *M. piperita, T. involucrate* and *U. massaica* exhibited antimicrobial activity at various degrees against seven tested Gram-negative bacterial and two fungal species namely *S. kisarawe, S. typhi, E. coli, K. oxytoca, P. aeruginosa, K. pneumonia, P. mirabilis, C. neoformans* and *C. albicans* respectively.

Keywords: Antimicrobial; Mentha piperita; Tragia involucrata (L); Urtica massaica.

1. INTRODUCTION

Antibiotic resistant organisms are serious growing medical problem caused by microbes who shared the same ecosystem worldwide [1]. The microorganisms are widely distributed from various environment including fecal coliform, hospitals, industries, water bodies and in contamination pathways [2-6]. The misuse of convectional antimicrobial agents have been a major problem towards human health since it causes severe health problems as the microbes undergoes chromosomal mutation to prevent or neutralize the drugs [7-9]. Examples of these microbes include Gram negative bacteria and some fungal species such as *Salmonella kisarawe, Salmonella typhi*, *Escherichia coli*, *Klebsiella oxytoca* [10] *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis, Cryptococcus neoformans* and *Candida albicans* [11]. However, these microbes are currently treated by synthetic antimicrobial agents developed from drug templates of plant origins. Examples of these plants include *Mentha piperita, Tragia involucrate* and *Urtica massaica.*

The *M. piperita* have been used by local practitioners particularly in Eastern and Western African countries as aromatic, anti-diarrhea, anti-septic, anticancer, indigestion and sore throat [12]. Previous study has shown that the species have been used by local communities in India to reduce lung cancer and mutagenicity [12]. Similarly, *T. involucrate* a plant species which belong to Euphorbiaceae family have long history of being used by many communities worldwide for the management of various ailments such as diarrhea, abdominal pain, hemolytic uremic syndrome infections, headache, eczema and wounds [13]. As regard *U. massaica* (Urticaceae), a flowering plant which is native in Congo, Burundi, Rwanda, Kenya, Uganda and Tanzania have also long history of being used in the treatment of urine blockage [14]. In Kenya, the plant is traditionally used in circumcision ceremonies. In this way, the plant is cut and placed in a sting line of two rows and boys who are to be circumcised run through it several times until the whole body is numb and ready for the ritual [14].

It is therefore justified that these medicinal plants (*M. piperita, T. involucrate* and *U. massaica*) have been traditionally used by local practitioners particularly in African countries for the management of various diseases [15-17]. In view of the above, searching for new antimicrobial agent from plant origin to prevent these diseases is of great importance [18,19]. This study therefore reports the antimicrobial activity of three herbal plants namely *M. piperita, T. involucrate* and *U. massaica* against seven Gram-negative bacteria. These include *S. kisarawe, S. typhi, E. coli, K. oxytoca, P. aeruginosa, K. pneumonia, P. mirabilis* and and two fungal species which are *C. neoformans* and *C. albicans*.

2. MATERIALS AND METHODS

2.1 Acquisition of Materials

Methanol and chloroform were purchased from Avantor Perfomance Materials Limited, Gujarat, India. Dimethyl sulphoxide (DMSO) and ethyl acetate were bought from RFCL Limited, Haryana, India. Nutrient broth and Nutrient agar were supplied by HIMEDIA Laboratories Pvt. Limited, Mumbai, India. Gram negative bacteria strains namely *S. kisarawe* (clinical isolate), *S. typhi* (NCTC 8385), *E. coli* (ATCC 25922), *K. oxytoca* (clinical isolate), *P. aeruginosa* (ATCC 29953), *K. pneumonia* (ATCC 700603), *P. mirabilis* (NCTC 1075) and two fungal species which are *C. neoformans* (clinical isolate) and *C. albicans* (ATCC 90028) were obtained from the department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS) in Tanzania. Gentamycin and iodonitrotetrazolium chloride were supplied by Mediatech Incorporation, Manassas, USA and SIGMA® (Sigma- Aldrich®, St. Louis, USA) respectively.

2.2 Preparation of Plant Extracts and Extraction

The plant materials which included leaves were collected from different parts of northern Tanzania. These included King'ori, Leguruki, Mulala, Kimundo and Kikatiti villages. Plant

species were identified by Mr. Emmanuel Mboya, a botanist from Tropical Pesticide Research Institute (TPRI) and voucher specimens coded MP-001, TI-002 and UM-003 for *M. piperita, T. involucrate* and *U. massaica* are kept at the University of Dodoma (UDOM). Plant materials were air dried under the shade and pulverized into fine particles using electric blender. Pulverized materials (500 g) were successively macerated in chloroform, ethyl acetate and methanol for 48 hours. The respective extracts were filtered through Whatman No. 1 filter paper on a plug of glass wool in a glass column and solvents were evaporated through the vacuum using a rotary evaporator and stored in a deep freezer at -20℃.

2.3 Determination of Antimicrobial Activity

Minimum inhibitory concentration of plants extracts against the tested bacterial and fungal species was determined by using 96 wells micro dilution method.

3. RESULTS

The study evaluated antimicrobial activity of leaf extracts from these herbal plants against seven Gram-negative bacterial and two fungal species. Findings presented as minimum inhibition concentrations (MIC) indicated that at least one tested organism was inhibited by plant extracts at MIC value between 1.56 and 3.15 mg/mL as summarized in Table 1.

The *M. piperita* chloroform leaf (MPCL) extract exhibited antimicrobial activity against *K. oxytoca, S. kisarawe, P. mirabilis* and *C. neuforman* with narrow MIC range of 1.56 and 3.15 mg/mL. It can be further noted from Table 1 that *M. piperita* ethyl acetate leaf (MPEL) exhibited antimicrobial activity against only two microbes namely *S. typhi* and *C. neuforman* with MIC value of 3.15 mg/mL. The *M. piperita* methanol leaf (MPML) extract exhibited antimicrobial activity against *S. typhi* and *E. coli* with MIC values of 3.12 and 1.56 mg/mL respectively.

As regards *T. involucrate,* the chloroform (TICL) and ethyl acetate leaf (TIEL) extract had high antimicrobial activity which is evidenced by MIC value of 1.56 mg/mL against *S. typhi, C. neuforman* and *P. aeruginosa* followed by *T. involucrate* methanol leaf (TIML) extract with MIC value of 3.12 mg/mL against *S. kisarawe, K. pneumonia, C. neuforman* and *C. albicans.* Antimicrobial investigation of *U. massaica* revealed that, chloroform (UMCL) and ethyl acetate leaf (UMEL) extracts displayed antimicrobial activity against *K. oxytoca* and *E. coli* at MIC value of 1.56 mg/mL. Apparently, ethyl acetate leaf (UMEL) extract exhibited MIC value of 3.12 mg/mL against *S. typhi* and *C. neuforman* which was less active compared to chloroform and methanol extracts.

4. DISCUSSION

Drug resistance among bacterial and fungal species is a major public health concern, [20] thus the discovery and development of new antimicrobial drugs from medicinal plants is among the most exciting areas for pharmacological research [21]. That is why this study is reporting antimicrobial activity of three commonly medicinal plants namely *M. piperita, T. involucrate* and *U. massaica* growing in Tanzania. Furthermore, the study evaluated leaf extracts from the studied herbal plants against seven pathogenic bacteria and two fungal species. Generally, the extracts demonstrated relatively high antibacterial and antifungal activity against tested seven Gram-negative bacteria and two fungal species, namely *S. kisarawe*, *S. typhi*, *E. coli*, *K. oxytoca*, *P. aeruginosa*, *K. pneumonia*, *P. mirabilis*, *C. neoformans* and *C. albicans* respectively.

The antimicrobial activity of *M. piperita* leaf extracts demonstrated activity against *S. kisarawe*, *K. oxytoca*, *P. mirabilis, C. neoformans, S. typhi* and *E. coli* with MIC range of 1.56 and 3.15 mg/mL. These findings imply that leaves of *M. piperita* are potential sources of drug leads for the management of diseases caused by these pathogenic microbes. Findings from this study are however in agreement with previous study conducted by Kuppusamy(2016) [22] in India who reported strong antibacterial activity of *M. piperita* aqueous leaf extracts against a range of pathogenic bacterial species such as *Bacillus subtilis, Pseudomonas aureus*, *P. aerogenosa, Serratia marcesens* and *Streptococcus aureus.* Another study conducted by Soković [23] on phytochemical analysis of *M. piperita* observed a high concentration of chemical composition such as oil methanol, menthyl acetate and menthone in the leaf extract of the plant. In addition, this extract found to possess antimicrobial activities.

Table 1. Antimicrobial activity of leaf extracts from *M. piperita*, *T. involucrate* and *U. massaica* against various microorganisms

Extract	Minimum inhibitory concentrations (MICs) in mg/mL								
	S. kisarawe	***S. typhi***	***E. coli***	***K. oxytoca***	***P. aeruginosa***	***K. pneumonia***	***P. mirabilis***	***C. neuforman***	***C. albicans***
MPCL	3.12	6.25	6.25	1.56	12.5	6.25	3.12	3.12	6.25
MPEL	6.25	3.12	12.5	12.5	12.5	6.25	6.25	3.12	12.5
MPML	6.25	3.12	1.56	6.25	12.5	12.5	6.25	6.25	12.5
TICL	6.25	1.56	12.5	12.5	12.5	6.25	6.25	1.56	3.12
TIEL	6.25	1.56	3.12	6.25	1.56	3.12	6.25	6.25	6.25
TIML	3.12	6.25	6.25	12.5	6.25	3.12	6.25	3.12	3.12
UMCL	3.12	6.25	6.25	1.56	12.5	6.25	3.12	3.12	6.25
UMEL	6.25	3.12	12.5	12.5	12.5	6.25	6.25	3.12	12.5
UMML	6.25	3.12	1.56	6.25	12.5	12.5	6.25	6.25	12.5
GENT	0.09	0.09	0.09	0.39	0.09	0.09	3.12	NA	NA
FLUC	NA	NA	NA	NA	NA	NA	NA	1.56	0.78

Key: GENT= Gentimicin, FLUC=Fluconazole, MPCL= M. piperita chloroform leaf extract, MPEL= M. piperita ethyl acetate leaf extract, MPML= M. piperita methanol leaf extract, TICL= T. involucrate chloroform leaf extract, TIEL= T. involucrate ethyl acetate leaf extract, TIML= T. involucrate methanol leaf extract, UMCL= U. massaica chloroform leaf extract, UMEL= U. massaica ethyl acetate leaf extract, UMML= U. massaica methanol leaf extract

In this study, it was further observed that *T. involucrate* chloroform (TICL) and ethyl acetate leaf (TIEL) extracts highly inhibited the growth of *S. typhi, C. neuforman and P. aeruginosa* respectively with MIC value of 1.56 mg/mL. From these results, it is therefore assumed that non-polar secondary metabolites including flavonoids, steroids, saponins, alkaloids, tannins and terpenoides which have been earlier reported to be found in leaf extract of *T. involucrata* are responsible for the activity displayed [21]. The present study results are in accordance with the results of Dsouza [24] who reported significant antibacterial activity of ethanolic leaf extract of the same plant species against *P. mirabilis* and *E. coli.*

The *U. massaica* chloroform (UMCL) and ethyl acetate leaf (UMEL) extracts selectively inhibited the growth of *K. oxytoca* and *E. coli* at MIC value of 1.56 mg/mL. Likewise, *U. massaica* ethyl acetate leaf (UMEL) extract had good antimicrobial activity against *S. typhi* and *C. neuforman* (MIC value of 3.12 mg/mL). The sensitivity of *U. massaica* extracts against these microbes might be due to the presence of menthone compound in the leaves of the plant. This compound along with other phytochemicals found in *U. massaica* exhibited antimicrobial activities [25].

5. CONCLUSION

The extracts from *M. piperita, T. involucrate* and *U. massaica* exhibited antimicrobial activity at various degrees against seven tested Gram-negative bacterial and two fungal species namely *S. kisarawe, S. typhi, E. coli, K. oxytoca, P. aeruginosa, K. pneumonia, P. mirabilis, C. neoformans* and *C. albicans* respectively. Therefore the use of plant materials as alternative to antimicrobial resistant pathogenic microbes is a novel finding. However, this study calls for further investigation on the compounds which possess high antimicrobial activity so that they can be isolated to increase the speed of drug discovery on the multi-drug resistance microbes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chowdhury PR, et al. *G*enomic interplay in bacterial communities: implications for growth promoting practices in animal husbandry. Frontiers in Microbiology. 2014;5.
2. Sales-Ortells H, Medema G. Screening-level microbial risk assessment of urban water locations: A tool for prioritization. Environmental Science & Technology. 2014;48(16):9780-9789.
3. Zhang XX, Zhang T, Fang HH. Antibiotic resistance genes in water environment. Applied Microbiology and Biotechnology. 2009;82(3):397-414.
4. Wellington EM, et al. The role of the natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. The Lancet Infectious Diseases. 2013;13(2):155-165.
5. Glassmeyer ST, et al. Transport of chemical and microbial compounds from known wastewater discharges: Potential for use as indicators of human fecal contamination. Environmental Science & Technology. 2005;39(14):5157-5169.
6. Ritter KS, Paul Sibley, Ken Hall, Patricia Keen, Gevan Mattu, Beth Linton, Len. Sources, pathways, and relative risks of contaminants in surface water and groundwater: a perspective prepared for the Walkerton inquiry. Journal of Toxicology and Environmental Health Part A. 2002;65(1):1-142.
7. Tomson G, Vlad I. The need to look at antibiotic resistance from a health systems perspective. Upsala Journal of Medical Sciences. 2014;119(2):117-124.
8. Wiegers AK, et al. Identification of a neutralizing epitope within antigenic domain 5 of glycoprotein B of human cytomegalovirus. Journal of Virology. 2015; 89(1):361-372.
9. Ahmad I, Aqil F. *In vitro e*fficacy of bioactive extracts of 15 medicinal plants against ESβL-producing multidrug-resistant enteric bacteria. Microbiological Research. 2007;162(3):264-275.

10. Masimba PJ, et al. Biological potentials of extracts and compounds from *Mammea usambarensis* verdc fruit. Journal of Advanced Scientific Research. 2014;5(3).
11. Wilson E, Chacha M, Omolo J. Antimicrobial and cytotoxic activities of extracts from *Sterculia quinqueloba* (Garcke) K. Schum and *Canthium crassum* Hiern. Journal of Pharmacognosy and Phytochemistry. 2014;3(3):125-129.
12. Rekha S. Comparative *in vitro* anthelmintic activity of chloroform and acetone extracts of *Mentha piperita.* International Journal of Pharmaceutical & Biological Archive. 2011;2(3).
13. Silja V, Varma KS, Mohanan K. Ethnomedicinal plant knowledge of the *Mullu kuruma* tribe of Wayanad district, Kerala. Indian Journal of Traditional Knowledge. 2008;7(4):604-612.
14. Amuka O. Efficacy of selected medicinal plants used by the ogiek communities against microbial related infections; 2014.
15. Ahmed HM. Ethnopharmacobotanical study on the medicinal plants used by herbalists in Sulaymaniyah Province, Kurdistan, Iraq. Journal of Ethnobiology and Ethnomedicine. 2016;12(1):8.
16. Rehecho S, et al. Ethnopharmacological survey of medicinal plants in Nor-Yauyos, a part of the landscape reserve Nor-Yauyos-Cochas, Peru. Journal of Ethnopharmacology. 2011;133(1):75-85.
17. Rajendran T. Biological resource base for traditional medicines. Health, Nature and Quality of Life. p. 41.
18. Vale PF, et al. Beyond killing can we find new ways to manage infection? Evolution, Medicine, and Public Health. 2016;1:148-157.
19. Spellberg B, et al. The epidemic of antibiotic-resistant infections: A call to action for the medical community from the Infectious Diseases Society of America. Clinical Infectious Diseases. 2008;46(2): 155-164.
20. Elisha IL, et al. The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine. 2017;17(1):133.
21. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564-582.
22. Kuppusamy P, et al. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications–An updated report. Saudi Pharmaceutical Journal. 2016;24(4):473-484.
23. Soković MD, et al. Chemical composition of essential oilsof thymus and mentha speciesand their antifungal activities. Molecules. 2009;14(1):238-249.
24. Dsouza D, Nanjaiah L. Antibacterial activity of 3, 3′, 4′-Trihydroxyflavone from Justicia wynaadensis against diabetic wound and urinary tract infection. Brazilian Journal of Microbiology; 2017.
25. Mulyangote LT. Ethnobotany and bioactivity of medicinal plants used to treat symptoms associated with gastro-intestinal infections in Namibia. University of Namibia; 2016.

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