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Antifungal and Cytotoxicity Activity of Plants Used as Herbal Teas in Tanzania

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Authors' contributions

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

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ABSTRACT

Aim: The current study was designed to determine antifungal and cytotoxicity activity of *Rhus vulgaris*, *Sphaeranthus bullatus*, *Osyris lanceolata*, *Ocimum gratissimum, Cymbopogon citratus, Acacia nilotica* and *Tylosema fassoglensis* used as herbal teas in Tanzania.
Study Design: *In vitro* antifungal assay and brine shrimp lethality test for cytotoxicity study.
Methodology: Broth dilution using 96-well micro dilution method was used in antifungal assay. Extracts were loaded in the wells of the first row, followed by serial dilution and 50 µl of the fungal suspensions (0.5 MacFarland standard turbidity) were added in each well. The first concentration which showed no fungal growth was considered as Minimum Inhibition Concentration (MIC). On the other hands Brine shrimp lethality test were employed to determine cytotoxicty activity. Ten brine shrimp were added to vials contains different concentration (240, 120, 80, 40, 24 and 8 µg/mL) of the extracts dissolved in DMSO. Each concentration was tested in duplicate to establish the number of surviving brine shrimp.
Results: Antifungal activity was demonstrated by all extract with the minimum inhibition concentration (MIC) value range from 1.56 mg/mL – 25 mg/mL against *Candida albicans* and

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Cryptococcus neoformans. The later fungus was susceptible to extracts tested compared to the former. Of the all extract tested 18% were non toxic against *Artemia salina* with lethal concentration (LC_{50}) value above 100 µg/mL. The highest cytotoxicity was exhibited by *Acacia nilotica* ethyl acetate root extract with LC_{50} 0.57 µg/mL.

Conclusion: Results of this study showed that extracts tested demonstrate moderate to weak antifungal activity and strong to weak cytotoxicity effects. Further study is required on these plant extracts to characterize bioactive compounds responsible for observed activities. Moreover, further toxicological studies are required in order to establish these plants as herbal teas which can be used for both refreshment and management of diseases.

Keywords: Antifungal; cytotoxicity; herbal tea; minimum inhibitory concentration.

1. INTRODUCTION

Herbal teas are among the fastest growing industry in the world due to neutraceutical potentials and the fact that most of them are free from caffeine contrast to black or green teas made from *Camellia sinensis* plant. They are consumed as beverage for refreshment or specific health promotion [1]. The most popular plants used as herbal teas in the world are hibiscus, ginger, chamomile and rose teas. Besides of these popular plant used as herbal teas, some ethnic groups in Tanzania have their own herbal teas made from plant species such as *Rhus vulgaris* (Anacardiaceae), *Sphaeranthus bullatus* (Asteraceae), *Osyris lanceolata* (Santalaceae), *Ocimum gratissimum* (Lamiaceae), *Cymbopogon citratus* (Gramineae), *Acacia nilotica* (Mimosaceae) and *Tylosema fassoglensis* (Caesalpiniaceae). These teas are used either as refreshment by people avoiding black tea, although they are also used for medicinal purposes [2,3]. Besides the use of these plants as herbal tea in Tanzania, They are also used for different purposes for instance *R. vulgaris* and *O. lanceolata* are used by some community in Uganda, Tanzania and Kenya to treat sexual diseases [2,3], *S. bullatus* was revealed by Machumi and coworkers [4] to posses compounds with antimalarial activity whilst *O. gratissimum*, *A niliotica* and *T. fassoglensis* were found to have antibacterial activity [3,5,6].

Consumption of tea in Tanzania is very high and due to this habit, plants used as herbal tea can be effectively utilized in easing diseases such as oral candidiasis. This calls for the need to determine antifungal activity of the plants commonly used as herbal tea in Tanzania. Thus this paper reports antifungal and cytotoxicity effects of *R. vulgaris, S. bullatus*, *O. lanceolata*, *O. gratissimum, C. citratus, A. nilotica* and *T. fassoglensis* used as herbal teas by ethnic groups in Tanzania.

2. MATERIALS AND METHODS

2.1 Plant Materials

The plant materials were collected from October – November 2015 based on the information available in traditional knowledge. *R. vulgaris* and *T. fassoglensis* were collected from Rorya district, *C. citratus, A. nilotica* and *O. gratissimum* were collected from Siha districts, *S. bullatus* and *O. lanceolata* were collected from Arumeru and Same districts respectively. The collected plants were identified by Daniel Sitoni a taxonomist in the herbarium department of the Tropical Pesticide Research Institute (TPRI) in Arusha, Tanzania. Voucher specimens number AN1, OG2, CC3, SB4, RV65, TF6 and OL7 were deposited at Nelson Mandela African institution of Science and Technology (NM-AIST), Arusha Tanzania.

2.2 Chemicals, Reagents and Strains Used

Dichloromethane was purchased from LOBA® (Lobachemia laboratory reagents, India). Ethyl acetate was purchased from RFCL Limited, India. Dimethyl sulfoxides (DMSO) were purchased from AVANTOR® (Avantor performance material limited, India). Saboraud dextrose and sabouraud dextrose broth were purchased from HIMEDIA® (Himedia Laboratories pvt Limited India). Iodonitrotetrazolium chloride (INT) and Fluconazole were purchased from SIGMA® (sigma –UK). Fungal strains namely *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (clinical isolate), and brine shrimp eggs were obtained from Department of Microbiology and Immunology, School of Medicine at Muhimbili University of Health and Allied Sciences.

2.3 Preparation and Extraction of Plant Material

Leaves, stem barks, roots and tuber of the plant species were air dried under the shade and then pulverized into fine particles. 250 g of pulverized materials were sequentially macerated using dichloromethane and ethyl acetate for 48 h, filtered using Whitman paper number 1 and solvents removed under *vacuo* using a rotary evaporator. 250 g of pulverized plant materials were also soaked in boiled water (1500 mL) maintained at 60℃ in the incubator for 24 h. The extract were sieved and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and then filtered using whatman paper 1. The process of centrifuge and filtration were repeated two times and final supernatant were collected and dried by freeze drier to eliminate water by sublimation. All extracts were stored in the deep freezer at -20℃ for further activity.

2.4 Test for Antifungal Activities

Minimum inhibitory concentrations (MICs) were created by serial micro dilution in duplicate using 96-well micro titer plates [7]. Initially, plates were loaded with 50 µL of sabourauds dextrose broth in each well, followed by an addition of 50 µL of the extract (100 mg/mL) in the first wells of each row tested to make a total volume of 100 µL in the first wells. After thorough mixing, 50 µL was drawn from each of the first row wells and placed into the next row of wells. This process was repeated down the columns to the last wells, where 50 µL was discarded. Thereafter, 50 µL of fungal suspension (approximately 0.5 McFarland standard turbidity) was then added to each well to make the final volume of 100 µL per well. The rows with fluconazole 2 mg/mL were used as standard positive controls, with DMSO (0.6%) as negative control and a row with broth and fungi used only to monitor bacterial growth. After serial micro-dilution, plates were incubated for 24 h at 37℃. MICs were determined by adding 20 µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation at 37℃ for 24 h. Fungal growth was indicated by a pink color. The lowest concentration that showed no fungal growth was considered as minimum inhibition concentration (MIC).

2.5 Brine Shrimp Test

Brine shrimp lethality test [8] was employed to determine assay of each extract. Stock solutions (40 mg/mL) of each extract were prepared by dissolving them in DMSO (0.6%). Artificial sea water was prepared by dissolving sea salt (3.8 g) in distilled water to make a concentration of 3.8 g/L [9]. The prepared solution was placed into a container partitioned into two parts with one side darkened. The shrimp eggs were spread in the dark side of the container and a lamp shone on the other side to attract hatched shrimps. After 48 h of hatching, ten brine shrimp (mature nauplii) were added to vials containing different concentration (240, 120, 80, 40, 24 and 8 µg/mL) of the extract drawn from the stock solution. The volume was then adjusted to 5 mL with artificial sea water. Each level of concentration was tested in duplicate. The negative control contained brine shrimp, artificial sea water and DMSO (0.6%) only. Cyclophosphamide (CPMD) injection was used as positive control. The vials were incubated and the number of surviving shrimp established after 24 h of incubation. Percentage of mortality was determined using statistical analysis.

Mortality (%) = (number of dead Nauplii/ initial number of live Nauplii) x 100 [10].

2.6 Statistical Analysis

Microsoft Excel (2007) was used to analyze data from the brine shrimp lethality test. Mortality rate was graphed against the log concentration and the line of best fit obtained. A regression equation was then used to calculate the LC_{50}, LC_{16}, LC_{84}, 95% confidence interval and regression coefficient [11].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Antifungal activity

Extracts from seven plants used as herbal tea in Tanzania were evaluated for antifungal properties against *Candida albicans* and *Cryptococcus neoformans* using serial micro-dilution method. Fluconazole, standard antifungal agent was employed as a positive control. The tested plant extracts extracted with dichloromethane, ethyl acetate and aqueous extracts demonstrated antifungal activity with the minimum inhibition concentration (MIC) values range of 1.56 to 25 mg/mL against *Candida albicans* and *Cryptococcus neoformans* (Table 1). It was found that 7.7% of extracts exhibited antifungal activity with MIC value 1.56 mg/mL. The remaining extracts exhibited

antifungal activity ranging from 3.13 mg/mL - 25 mg/mL. The antifungal activity varied between plant species with the order of *T. fassoglensis* > *R. vulgaris* > *O. lanceolata* > other species.

Table 1. Antifungal activity of *R. vulgaris*, *A. nilotica, T. fassoglensis*, *O. gratissimum*, *S. bullatus*, *O. lanceolata*, *C. citratus* and *C. sinensis* extracts

Plant extracts	MIC value (mg/mL)	
	C. albicans	***C. neoformans***
RVBD	6.25	12.5
RVBE	25	12.5
RVBA	3.13	3.13
RVLD	3.13	3.13
RVLE	3.13	1.56
RVLA	12.5	3.13
RVRD	3.13	25
RVRE	12.5	6.25
RVRA	6.25	1.56
ANBD	6.25	12.5
ANBE	25	3.13
ANBA	12.5	12.5
ANRD	6.25	3.13
ANRE	6.25	6.25
ANRA	12.5	6.25
TFLD	6.25	3.13
TFLE	3.13	1.56
TFLA	3.13	1.56
TFRD	12.5	12.5
TFRE	3.13	1.56
TFRA	6.25	3.13
OGLD	3.13	3.13
OGLE	12.5	6.25
OGLA	12.5	3.13
OGFD	6.25	12.5
OGFE	25	12.5
OGFA	12.5	12.5
OGRD	3.13	3.13
OGRE	3.13	3.13
OGRA	12.5	6.25
SBD	12.5	3.13
SBE	6.25	6.25
SBA	12.5	12.5
OLD	25	12.5
OLE	6.25	6.25
OLA	1.56	6.25
CCD	12.5	6.25
CCE	6.25	6.25
CCA	6.25	3.13
FLUC	0.19	0.19

Key: RVBD: R. vulgaris stem bark dichloromethane, RVBE: R. vulgaris stem bark ethyl acetate, RVBA: R. vulgaris stem bark aqueous, RVLD: R. vulgaris leaf dichloromethane, RVLE: R. vulgaris leaf ethyl acetate, R. vulgaris leaf aqueous, RVRD: R. vulgaris root dichloromethane, RVRE: R. vulgaris root ethyl acetate, RVRA: Rhus vulgaris root aqueous, ANBD: A. nilotica stem bark dichloromethane, ANBE: A. nilotica stem bark ethyl acetate, ANBA: A. nilotica stem bark aqueous, ANRD: A. nilotica root dichloromethane, ANRE: A nilotica root ethyl acetate, ANRA: A. nilotica root aqueous, TFLD: T. fassoglensis leaf dichloromethane, TFLE: T. fassoglensis leaf ethyl acetate, TFLA: T. fassoglensis leaf aqueous, TFRD: T. fassoglensis root dichloromethane, TFRE: T. fassoglensis root ethyl acetate, TFRA: T. fassoglensis root aqueous, OGLD: O. gratissimum leaf dichloromethane, OGLE: O. gratissimum leaf ethyl acetate, OGLA: O. gratissimum leaf aqueous, OGFD: O. gratissimum flower dichloromethane, OGFE: O. gratissimum flower ethyl acetate, OGFA: O. gratissimum aqueous, OGRD: O. gratissimum root dichloromethane, OGRE: O. gratissimum root ethyl acetate, OGRA: O. gratissimum root aqueous, SBD: S. bullatus dichloromethane, SBE: S. bullatus ethyl acetate, SBA: S. bullatus aqueous, OLD: O. lanceolata dichloromethane, OLE: O. lanceolata ethyl acetate, OLA: S. bullatus aqueous, CCD: C. citratus dichloromethane, CCE: C. citratus ethyl acetate, CCA: C. citratus aqueous, CSD: C. sinensis dichloromethane, CSE: C. sinensis ethyl acetate, FLUC: Fluconazole

Cryptococcus neoformans was found to be more susceptible to the extracts than *Candida albicans*. The former fungal species was susceptible to 12.8% of the extracts which exhibited MIC value of 1.56 mg/mL. These extracts are *R. vulgaris* leaf ethyl acetate extract (RVLE), *R. vulgaris* roots aqueous extract (RVRA), *T. fassoglensis* leaf ethyl acetate extract (TFLE), *T. fassoglensis* roots ethyl acetate extract (TFRE) and *T. fassoglensis* leaf aqueous extract (TFLA). The antifungal activity of *O. lanceolata* (OLA) was four times more active against *C*. *albicans* as compared to the activity exhibited against *C*. *neoformans*. *Candida albicans* which is known as causative agent of oral and vaginal candidiasis especially to individuals with uncompromised immune system was resistant to the tested extracts compared to *C. neoformans*. This study showed that only OLA was active to the former fungal species with the MIC value 1.56 mg/mL.

Present study revealed that dichloromethane extracts has less antifungal activity compared to aqueous and ethyl acetate extracts. It was observed that three aqueous extracts (OLA, RVRA, and TFLA) and three ethyl acetate extracts (RVRE, TFLE and TFRE) showed antifungal activity with MIC value 1.56 mg/mL while all of the dichloromethane extracts exhibited activity higher than 1.56 mg/mL.

3.1.2 Cytotoxicity study

The brine shrimp lethality assay has been used for several years as the simple, rapid, robust and inexpensive method for preliminary bioassay technique to screen cytotoxicity of the plant extracts [12]. This assay was employed in the evaluation of plant parts used as herbal teas by ethnic groups in Tanzania. They exhibited cytotoxicity activity against brine shrimp larvae with LC_{50} range of 0.57 – 1,180.79 µg/mL (Table 2). The most cytotoxic activity was found in ANRE with LC_{50} 0.57 µg/mL. It was evident that 17.96% of the extracts (RVLD, TFLD, TFLE, TFRD, OGFD, and OGFE) in these finding had LC_{50} above 100 µg/mL suggested that they are non toxic. Potential toxicity was considered to extracts with LC_{50} values below100 µg/mL. Toxicity of extracts with LC_{50} <100 µg/mL were categorized as mildly toxic (LC_{50} >30 <100 µg/mL), moderately toxic (LC_{50} 10 - 30 µg/mL), toxic (LC_{50} 1 - 10 µg/mL) and highly toxic (LC_{50} <1). Therefore in this study 23.08% of the extracts (OLA, SBD, OLD, OGRA, OGRE, OGLE, OGLD, ANBE and RVBA) were mildly toxic, 28.2% moderately toxic (CCE, SBA, SBE, TFRE, ANRA, ANBA, RVRA, RVRE, RVLE andRVRD), 28.2% (RVBE, RVLA, RVRD, ANBD, ANRD, TFLA, TFRA, OLE, CCD and CCA) were toxic and 2.56% (ANRE) were highly toxic.

Present study observed that cytotoxicity of plant extracts increase with increase of polarity. It was emanated that 20.51% of plant extracts exhibited cytotoxicity activity with LC_{50} < than 20 µg/mL was aqueous extracts followed by ethyl acetate extracts which exhibited 17.13% and dichloromethane extracts which exhibited 5.13% with LC_{50} less than 20 µg/mL.

3.2 Discussion

Natural products have been used for long time for refreshment, treatment and as cosmetics [13]. Among the widely used natural product is herbal tea. Herbal teas are used due to their refreshment potentials, attractive aroma and potential health effect [1]. In the indigenous health system, plants used as herbal teas have been applied for prevention and treatment of diseases. However, not all plants used as herbal teas in the communities are safe to be consumed frequently by human. In this study, the antifungal and cytotoxicity effect of seven plants used as herbal teas by different ethnic groups were evaluated. The selection of these plants was based on the information obtained from the ethnic groups in Tanzania.

The result from this study exhibited antifungal activity from MIC value of 1.56 – 25 mg/mL. Several studies are available showing efficacy of plant extracts as antifungal agents [14]. According to Aligiannis et al. [15] MIC values of crude extracts lower than 0.5 mg/mL are considered to show strong inhibition, 0.5 – 1.5 mg/mL moderate inhibition, and values from 1.6 mg/mL and above show weak inhibition. In the drug delivery MIC value of plant extract greater than 1 mg/mL are considered of no significance, however they should also reported as they can be incorporated with other extract to improve biological values [16]. However, in this study 7.7% of the tested extracts (RVLE, RVRA, TFLE, TFLA and OLA) exhibited antifungal activity with MIC value 1.56 mg/mL which were categorized by Aligiannis and coworkers [15] as moderately active. Studies by [17,18] reported that inhibitory potential of the plant extracts depends on the type of plant and method of extraction. Similar result was obtained by the present study. *T. fassoglensis* and *R. vulgaris* species were

exhibited higher antifungal activity against *C. neoformans* and *O. lanceolata* showed higher activity against *C. albilcans*. It was also revealed that aqueous and ethyl acetate extracts had higher antifungal activity than dichloromethane extracts.

Table 2. Cytotoxicity effect of *R. vulgaris*, *A. nilotica*, *T. fassoglensis, O. gratissimum*, *O. lanceolata*, *S. bullatus* and *C. citratus* extracts

Plant extract	Regression equation	LC_{50} (µg/mL)	95% Confidence interval (µg/mL)	R^2
RVBD	y = 17.40x + 37.32	5.35	1.64 - 17.44	R^2 = 0.88
RVBE	y = 35.86x + 1. 49	22.52	12.96 – 39.12	R^2 = 0.87
RVBA	y = 56.18x - 37.71	36.41	25.59 – 51.81	R^2 = 0.97
RVLD	y= 12.74 +13.48	734.06	109.30 – 4,929.95	R^2 = 0.75
RVLE	y = 31.23x + 32.81	3.55	2.20 – 5.73	R^2 = 0.82
RVLA	y = 24.52x + 20.04	16.68	6.19 – 44.90	R^2 = 0.98
RVRD	y = 19.13x + 23.73	23.62	6.53 – 83.62	R^2 = 0.94
RVRE	y = 24.27x + 33.15	4.94	1.97 – 12.39	R^2 = 0.97
RVRA	y = 35.99x + 19.67	6.97	3.81– 12.73	R^2 = 0.92
ANBD	y = 44.399x - 13.41	26.80	15.52 – 46.28	R^2 = 0.93
ANBE	y = 10.662x + 33.98	31.79	2.94 – 343.75	R^2 = 0.97
ANBA	y = 23.92x + 31.10	6.17	2.49 – 15.26	R^2 = 0.82
ANRD	y = 49.39x - 17.38	23.13	14.91 – 35.88	R^2 = 0.91
ANRE	y = 23.32x + 55.68	0.57	0.25 – 1.33	R^2 = 0.95
ANRA	y = 20.03x + 39.19	3.47	1.17 – 10.25	R^2 = 0.91
TFLD	y = 21.90x - 9.30	509.99	189.52 – 1372.38	R^2 = 0.88
TFLE	y = 25.93x - 3.21	112.68	44.20 – 287.28	R^2 = 0.98
TFLA	y = 50.92x - 20.31	24.03	16.29 – 35.44	R^2 = 0.97
TFRD	y = 44.71x - 3.23	203.66	65.92– 629. 21	R^2 = 0.81
TFRE	y = 22.71x + 30.02	7.58	3.17 – 18.14	R^2 = 0.96
TFRA	y = 71.61x - 39.15	17.57	12.53 – 24.66	R^2 = 0.91
OGLD	y = 38.07x - 24.579	90.98	51.46 – 16.85	R^2 = 0.86
OGLE	y = 17.19x + 16.98	83.37	23.61 – 294.37	R^2 = 0.96
OGLA	y = 30.57x + 23.452	7.39	3.63 – 15.01	R^2 = 0.96
OGFD	y = 31.53x - 29.038	321.30	168.84 – 611.43	R^2 = 0.83
OGFE	y = 28.19x - 16.087	220.97	93.44 – 522.60	R^2 = 0.99
OGFA	y = 48.88x - 20.483	27.44	17.61 – 42.748	R^2 = 0.90
OGRD	y = 21.16x - 15.001	1,180.79	423.68 – 3,290.86	R^2 = 0.75
OGRE	y = 49.045x - 33.8	51.15	32.87– 79.58	R^2 = 0.89
OGRA	y = 46.32x - 40.71	90.83	59.04 – 139.79	R^2 = 0.86
OLD	y =75.56x – 65.66	33.94	24.61 – 46.80	R^2 = 0.93
OLE	Y = 46.19x – 3.47	14.37	8.52 – 24.23	R^2 = 0.71
OLA	y = 86.49x- 104.8	61.64	47.97 – 79.20	R^2 = 0.76
SBD	y = 24.35x + 8.92	48.64	17.95 – 131.81	R^2 = 0.94
SBE	y = 33.45x + 35.55	2.70	1.17 - 6.25	R^2 = 0.99
SBA	Y = 30.14x + 29.30	4.86	2.43 – 9.73	R = 0.99
CCD	y = 54.91x - 30.52	29.27	20.41- 41.97	R^2 = 0.98
CCE	y = 48.71x + 20.34	4.06	2.29 – 7.22	R^2 = 0.98
CCA	y = 48.63x - 9.48	16.72	10.72 – 26.11	R^2 = 0.92
CPMD	y = 69.95x – 32.84	15.28	12.02 – 22.32	R^2 = 0.98

Key: RVBD: R. vulgaris stem bark dichloromethane, RVBE: R. vulgaris stem bark ethyl acetate, RVBA: R. vulgaris stem bark aqueous, RVLD: R. vulgaris leaf dichloromethane, RVLE: R. vulgaris leaf ethyl acetate, R. vulgaris leaf aqueous, RVRD: R. vulgaris root dichloromethane, RVRE: R. vulgaris root ethyl acetate, RVRA: Rhus vulgaris root methanolic, ANBD: A. nilotica stem bark dichloromethane, ANBE: A. nilotica stem bark ethyl acetate, ANBA: A. nilotica stem bark aqueous, ANRD: A. nilotica root dichloromethane, ANRE: A nilotica root ethyl acetate, ANRA: A. nilotica root aqueous, TFLD: T. fassoglensis leaf dichloromethane, TFLE: T. fassoglensis leaf ethyl acetate, TFLA: T. fassoglensis leaf aqueous, TFRD: T. fassoglensis root dichloromethane, TFRE: T. fassoglensis root ethyl acetate, TFRA: T. fassoglensis root aqueous,OGLD: O. gratissimum leaf dichloromethane, OGLE: O. gratissimum leaf ethyl acetate, OGLA: O. gratissimum leaf aqueous, OGFD: O. gratissimum flower dichloromethane, OGFE: O. gratissimum flower ethyl acetate, OGFA: O. gratissimum aqueous, OGRD: O. gratissimum root dichloromethane, OGRE: O. gratissimum root ethyl acetate, OGRA: O. gratissimum root aqueous, SBD: S. bullatus dichlorometahne, SBE: S. bullatus ethyl acetate, SBA: S. bullatus aqueous, OLD: O. lanceolata dichloromethane, OLE: O. lanceolata ethyl acetate, OLA: S. bullatus aqueous, CCD: C. citratus dichloromethane, CCE: C. citratus ethyl acetate, CCA: C. citratus aqueous, CPMD: Cyclophosphamide

C. albicans was resistant to most of extracts compared to *C. neoformans*. Resistance of *C. albicans* to antifungal drugs has been elaborated by several studies [14,19]. Mechanism of the resistance of *C. albicans* has been identified to be caused by alteration in the gene encoding the target enzymes ERG11or over expression of efflux pump genes including CDR1, CDR2, and MDR1 [20]. On the other hand *C. neoformans* which is the main cause of cryptococcal meningitis especially to HIV/AIDS patients has been explained to have only rare resistance to most of antifungal drugs tested [21].

It was interest of this study to determine cytotoxicity activity of these plants used as herbal teas using brine shrimp lethality test. The brine shrimp lethality test is considered as the inexpensive, rapid and simple bioassay for cytotoxicity study of the plant extracts [12]. According to Meyer and coworkers [8] plant extracts with cytotxicity value above LC_{50} <100 µg/mL are considered non toxic. In the present study brine shrimp findings were interpreted as LC_{50} <1.0 µg/mL – highly toxic; LC_{50}1.0 - 10.0 µg/ml – toxic; LC_{50} 10.0 - 30.0 µg/ml moderately toxic; LC_{50} >30 <100 µg/mL – mildly toxic, and > 100µg/ml as non - toxic [22]. The highest cytotoxicity with LC_{50} 0.57 µg/mL was found in ANRE. Out of 5 extracts that exhibited antifungal with MIC value of 1.56 mg/mL against *C. neoformans* and *C. albicans*, only TFLE displayed LC_{50} value higher than 100 µg/mL which is considered non toxic whilst OLA and TFLA exhibiting mildly and moderate toxicity respectively. It was however evident that 16 out of 39 tested plant extracts exhibited cytotoxicity with LC_{50} values below 20 µg/mL. In the cancer drug discovery research the extracts with cytotoxicity effect below LC_{50} 20 µg/mL are considered potential anticancer agents [23]. Hence 41% of the extracts in the current study are potential anticancer agents.

4. CONCLUSION

Findings from this study showed that extracts tested from plant used as herbal tea in Tanzania exhibited moderate to weak antifungal activity and strong to weak cytotoxicity activity. Further study is required on these plant extracts to characterize bioactive compounds responsible for observed activities. Moreover further toxicological studies such as acute, sub acute and chronic toxicity on mice is required in order to establish these plant as herbal teas which can be used for both refreshment and management of diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Inouye S, Takahashi M, Abe S. Inhibitory activity of hydrosols, herbal teas and related essential oils against filament formation and the growth of *Candida albicans*. Japaneese Journal of Medical Mycology. 2009;50(4):243-51.
2. Mothogoane MS. Osyrislanceolata: Santalaceae, Hochst. & Steud. South African National Biodiversity Institute's Plant Information; 2011.
(Accessed 31 may 2016)
Available:www.plantzafrica.com
3. Adongo JO, Omolo JO, Njue, AW, Matofari JW. Antimicrobial activity of the root extracts of *Tylosema fassoglensis* Schweinf. Torre & Hillc (Caesalpiniaceae). Science Journal of Microbiology; 2012.
DOI: 10.7237/sjmb/209
4. Machumi F, Yenesewa A, Midiwoa JO, Heydenreichb M, Kleinpeterb E, Tekwanic BL, et al. Antiparasitic and anticancer carvotacetone derivatives of *Spheranthus bullatus.* Natural Product Communication. 2012;7(99):1123-26.
5. Ali A, Akhtar N, Khan BA, Khan MS, Rasul A, Zaman E, et al. *Acacia nilotica*: A plant

of multipurpose medicinal uses. Journal of Medicinal Plants Research. 2012;6(9): 1492-6.
6. Mugisha KM, Origa OH. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. Afr. Health Sci. 2005; 5(1):40- 9.
7. Ellof JN. A quick microplate method to determine the minimum inhibitory sensitive and concentration of plant extracts for bacteria. Planta Medica. 1998;64:711-13.
8. Meyer B, Ferrigni N, Putnam J, Jacobsen L, Nichols D, McLaughlin J. Brine shrimp-A convenient general bioassay for active plant constituents. Planta Med. 1998;45: 31-34.
9. Ramachandran S, Vamsikrishna M, Gowthami KV, Heera B, Dhanaraju MD. Assessment of cytotoxic activity of *Agave cantula* using Brine shrimp (*Artemia salina*) lethality bioassay. Asian Journal of Scientific Research. 2011;4:90-94.
10. Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Medicinal Plants Research. 2010; 4:407-14.
11. Hamidi MR, Jovanova B, Panovska TK. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. Macedonian Pharmaceutical Bulletin. 2014;60(1):9–18.
12. Ji HF, Li XJ, Zhang HY. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? Embo Rep. 2009;10(3): 194-200.
13. Nashiela DF, Norihan A, Nooraain H, Azizah AH. Antioxidant activity of herbal tea prepared from *Cosmos caundatis*. Leaves at different maturity stage. IFRJ. 2015;22(3):1189-1194.
14. Hussaini JSA, Mohana AMGA. An evaluation of the antifungal activity of some local medicinal plants against growth of *Candida albicans in vitro*. AL-Qadisiya Journal of Vet. Med. Sci. 2010;9(2):60-68.
15. Aligiannis N, Kalpotzakis E, Mitaku S, Chinou, IB. Composition and antimicrobial activity of the essential oils of two *Origanum* species. J. Agric. Food Chem. 2001;40:4168-70.
16. Rios J, Recio M. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology. 2005;100(1):80-84.
17. Howard L, Pandjaitan N. Pressurized liquid extraction of flavonoids from spinach. J. Food Sci. 2008;73:151-7.
18. Palma M, Pineiro Z, Barroso CG. Stability of phenolic compounds during extraction with superheated solvents. J. Chromatogr. 2001;921:169-174.
19. Cowen LE, Anderson JB, Kohn LM. Evolution of drug resistance in *candida albicans*. Annu. Rev. Microbial. 2002;56: 139–65.
20. White TC, Holleman S, Dy F, Mirels LF, Stevens DA. Resistance mechanisms in clinical isolates of *Candida albicans*. Antimicrobial Agents Chemotherapy. 2002; 46(6):1704–13.
21. Archibald LK, Tuohy MJ, Wilson DA, Nwanyanwu O, Kazembe PN, Tansuphasawadikul S, Eampokalap B, Chaovavanich A, Reller LB, Jarvis WR, Hall GS, Procop GW. Antifungal Susceptibilities of *Cryptococcus neoformans*. Emerging Infectious Diseases. 2004;10(1):143-45.
22. Moshi MJ, Innocent E, Magadula JJ, Otieno DF, Wesheita A, Mbambazi K, Nondo RSO. Brine shrimp toxicity of some plants used as traditional medicines in Kagera Region, North Western Tanzania. Tanzania Journal of Health Research. 2010;12(1):1-6.
23. Hameed AES, Salih A, Bazaid SA, Shohayeb MM, El-Sayed MM, El-Wakil EA. Phytochemical studies and evaluation of antioxidant, anticancer and antimicrobial properties of *Conocarpus erectus* L. growing in Taif, Saudi Arabia. Eur J Med Plants. 2012;2:93–112.

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