Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.28, 2014

Bioactivity of crude extracts of Ascomycetes isolated from Tanzanian traditionally fermented milk, *Mtindi*

Jane S. Mlimbila[2], Masoud H. Muruke[1] Ken M.M.Hosea[1]
[1]University of Dar es Salaam, Department of Molecular Biology and Biotechnology,
College of Natural and Applied Sciences, Mwalimu J.K. Nyerere Mlimani Campus,
Uvumbuzi Road, P.O. Box 35179, Dar es Salaam, Tanzania; Tel: +255 22 2410 223.
Email: muruke@udsm.ac.tz;kenhosea2@gmail.com
[2]Muhimbili University of Health and Allied Sciences, Department of Environmental and Occupational Heath,
P.O. Box 65015, Dar es Salaam, Tanzania. Tel: +255 714 210174.
E-mail: jnmlimbila@yahoo.com

Abstract
In an attempt to find potential functional foods in Tanzania, a study was conducted to assess bioactivity of 18 ethyl acetate extracts from nine (9) Ascomycetes strains. Namely; *Candida tropicalis, C. pararugosa, Clavispora lusitaniae, Issatchenkia orientalis, Pichia kudriavzevii, Pichia guilliermondii, Galactomyces geotricum*, *Debaryomyces hansenii* and *Yarrowia lipolytica* isolated from traditional fermented milk "*mtindi*". Lethality test of the extracts was determined using *Artemia salina naupalii* in a Brine Shrimp Test (BST). The lethal concentration (LC_{50}) obtained ranged from 89.7µg/ml to over 1000 µg/ml. Bioactivity results showed that, one of the 18 extracts had exhibited a strong antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aureginosa* and *Vibrio cholera* having minimum inhibitory concentration (MIC) values of 0.1653mg/ml on each account. More than 40% of extracts exhibited strong to moderate antifungal activity against *Cryptococcus neoformans* (MIC 0.16 mg/ml – 1.25 mg/ml). In conclusion, these results suggest that yeasts found in traditional fermented milk have potential biological activity that could be used for treatment of some diarrhoeal and fungal infections and possibly tuberculosis.
Key words: Yeasts, Brine Shrimp Test, Antimicrobial Activity, Traditional Fermented milk

1. Introduction

Antibiotic resistant and infectious diseases are becoming a major public health concern worldwide. Misuse of antibiotics has turned out to be a common phenomenon leading to development of multi-drugs resistance against pathogens, which may eventually result in common infections becoming untreatable (Akabanda and Glover, 2010). Fermented dairy products have played an important role in human nutrition for centuries and when administered in adequate amount, they can confer a health benefit on the host (Stanton et al., 2005). Lactic acid bacteria (LAB) fermentation has a long history and reported as effective bio-preservation with abundant of literature on its antimicrobial activities against food borne pathogens (Yang et al., 2012; Mufandaedza et al.2006; Kuwabara et al., 1995).

"*Mtindi"* is a Tanzanian traditional fermented milk that is prepared by two major methods; first, is by spontaneously fermentation of raw milk at ambient temperature in different types of containers such as earthenware pots, gourds and in some semi-urban areas plastic containers, then covered and placed inside the hut or house to ferment for 24 to 36 hours. The containers are normally not sanitized after every fermentation cycle and are usually reused for the same purpose. The Maasai would store milk in gourds and chopped and burnt pieces of woods would be added to give it a smoky flavour. The milk usually fermented promptly after being added into the gourds (Isono and Shjngu, 1994).The second type of *mtindi* is produced through the addition of a portion of the remnant of previously fermentation into warm milk which was previously boiled (back-slopping). The inoculated milk is covered and left to ferment for about 12 hours.

Like most traditionally fermented products, the specific microflora in *mtindi* is unpredictable from one fermentation cycle to the next and therefore wavering in its quality (Lourens-Hattingh and Viljoen, 2002; Savova and Nikolova, 2002). Lactic acid bacteria (LAB), yeasts and coliforms have been found to coexists in traditionally fermented milk (Paucean et al., 2010; Gran et al., 2003; Narvhus, 2001). The low pH and the presence of antimicrobial substances from fermenters reckon traditional fermented milk safe (Samet-bali et al., 2012; Stanton et al., 2005). Both popular wisdom and some research findings have acclaimed fermented milk to

be more nutritious and health-promoting than fresh milk (Akabanda and Glover, 2010). The consumption of fermented dairy products was reported to have attributed to the longevity of Bulgarians (Yesillik *et al*., 2011; Stanton et al., 2005). Traditional fermented milk containing yeasts as part of microflora or starter culture has been associated with the treatment of colitis and diarrhoeal diseases in (Ni et al. 2007; Dixit and Gandhi, 2006; Mufandaedza et al., 2006; Jespersen, 2003) and even tuberculosis in Russia during post World War II era (Lopitz et al., 2006).

Yeasts are reported to play favourable effects in fermented milk over most bacteria due to low pH and secretion of bioactive substances (Lourens-Hattingh and Viljoen, 2002; Savova and Nikolova, 2002). Health benefits of traditional fermented milk can therefore be expressed either through the interaction of ingested live bacteria or yeasts with the host or is a result of ingestion of metabolites such as organic acids, antibiotic factors and volatile acids produced by fermenters during the fermentation process (Stanton et al., 2005). It can therefore be speculated that, the more diverse the yeasts are in fermented milk the higher is the potential of getting more bioactive metabolites against various food borne pathogens. However, to our knowledge a limited number of studies have investigated the antimicrobial activities of food-borne pathogens by natural yeasts isolated from dairy products and their mechanisms.

The aim of the present study was to evaluate the antimicrobial activity of crude extracts of yeasts isolated from traditional fermented milk against selected human pathogens, Gram-positive, Gram-negative bacteria, and against a fungus. Investigation was carried out to establish whether claimed therapeutic effect of traditional fermented milk against diarrhoea, venereal diseases and tuberculosis could be attributed to metabolites from yeasts found in traditional fermented milk.

2. Materials and Methods

2.1 Fermented milk sampling

Thirteen samples of traditional Tanzanian fermented milk, *mtindi* were purchased from local markets in Mwanza (Mw); n = 4) Dar es Salaam (Dar; n = 4), Morogoro (Moro; n=3), and Kilimanjaro (Kili; n=2) located in the North-western, Coastal, Eastern and Northern of Tanzania, respectively. *Mtindi* from Mwanza and Morogoro was prepared through spontaneous fermentation of raw milk at room temperature for 24 to 36 hours in non sanitized earthenware after previous fermentation, whereas, *mtindi* from Kilimanjaro and Dar es Salaam was made through addition of *mtindi* portions from previously fermentation in to warm, previous boiled milk and kept in well sanitized and covered plastic containers or buckets and left for 12 hours.

2.2 Yeasts isolation and preliminary identification

One gram of each yoghurt and 'mtindi' sample was diluted in 9 ml sterile 0.1% Ringer's solution (0.9 % NaCl, 0.042 % KCl, 0.48 % $CaCl_2.6H_20$, 0.02 % $NaHC0_3$, and 1 % Glucose) followed by decimal dilution up to 10^{-6} and 1ml was used for pour plating in duplicate on malt extract agar,(MEA), (HIMEDIA, India) at 30 ^oC for 24 hours. About 0.1 ml of 10^{-3}, 10^{-5} and 10^{-6} dilutions each was spread on MEA plates supplemented with Chloromphenicol (100mg/l) to inhibit bacterial growth then incubated at 30 ^oC for 24 to 48 hours (Memmert, Depex B.V, The Netherlands). Yeast isolates per plate were selected based on their microscopic appearance, morphological differences, color, margin and elevation of the colonies (Kavas et al., 2006) further sub-cultured for 12 hours at 30^oC on Malt extract broth supplemented with Chloromphenicol (100 mg/l). After 12hrs, broth cultures were then streaked on malt extract agar plates supplemented with Chloromphenicol (100 mg/l) for purification. The strains were then cultured on malt extract slants at 30 ^oC for 24 to 48 hours and kept at 4 ^oC (KIKSVV33A; Robert Bosch, Germany). Eighteen yeasts strains recorded as *Candida tropicalis* (CtMw6), *Candida pararugosa* (Cp1Mw3, Cp2Mw4, Cp3Mw5), *Pichia kudriavzevii* (PkMw1*), Clavispora lusitaniae (CllMw7)*, *Issatchenkia orientalis (IoMw2)*, (NDMw8), *Galactomyces geotricum* (Gg1Dar1, Gg2Dar2, Gg3Dar3, Gg4Dar4, Gg5Kili1, Gg6kili2), *Pichia guilliermondii (Pg1Moro, Pg2Dar5, Pg3Dar6)* as identified by Mlimbila *et al.* (2013) were studied .

2.3 Extraction of yeast secondary metabolites

The process of extracting yeast metabolites is summarized diagrammatically in Figure 1. A loopful of pure yeast colonies were cultured for in 5 ml Malt extract broth HIMEDIA (Himedia Laboratories Pvt Ltd., Mumbai, India)

and incubated 12 hours. After 12 hours, the culture was then introduced into 700 ml of sterile Malt extract broth and incubated at 30 ^{o}C for 14 days with shaking at 150 rpm only in the first 24 hours. After two weeks, yeasts cells were filtered out using whatman's filter paper (Sigma- Aldrich) and a filter funnel, leaving behind secondary crude metabolite in a filtrate. Metabolites were extracted by solvent extraction procedure using ethyl acetate (EtOAce) (Ranbaxy, New Delhi) as organic solvent. An equal volume of ethyl acetate and filtrate were taken in separating funnel and shaken vigorously by hands for about 45 minutes. The solution was left to stand until two distinct layers appeared. Ethyl acetate was evaporated using rotary evaporator at 40 ^{o}C, followed by vacuum drying to remove any remaining filtrate. Di-methyl sulphoxide (DMSO, Sigma Poole, Dorset, UK) was added to the extracts then stored at 0 ^{o}C in a refrigerator (KIKSVV33A; Robert Bosch, Germany) for further use.

Figure 1: Stages in extraction of crude extract using EtOAce (1) samples of traditional fermented milk, (2) purified yeasts isolate from fermented milk samples, (3) 14 days old malt extract broth yeast culture (Cells free) (4) Liquid-liquid extraction of 14 old yeast culture filtrate using EtOAceas a solvent (5) Rotary evaporator evaporating off the solvent EtOAce (6) crude extracts dissolved in DMSO.

2.4 Brine shrimp lethality test

The cytotoxicity activity of the extract was predicted using the brine shrimp lethality test (BST) as described by Meyer *et al*. (1982). Stock solutions of extracts were made by dissolving 20 mg of the crude extracts to 500 µl of DMSO to make up concentrations of 40 µg/ml each. Ten brine shrimp larvae were incubated in various extract concentrations (240, 120, 80, 40, 24 and 8 µg/ml) in duplicate vials for 24 hours (Brine shrimp eggs were purchased from Great Salt Lake, USA). Negative control was made by placing brine shrimps in a mixture of sea water and DMSO only. The average number of survived brine shrimps was determined by examining the vials after 24 hours against the lighted background. Probity analysis was performed using SPSS 15.0 software where by the regression equation was obtained and used to determine concentration of extract that killed 50% of the larvae (LC_{50}).

2.5 Minimum Inhibitory Concentrations (MIC) determination.

The extracts were tested for *in-vitro* antibacterial activity against six bacteria: *Staphylococcus aureus*, NCTC 6571, *Streptococcus faecalis* (clinical isolate), *Pseudomonas aureginosa* NCTC 10662, *Mycobacterium smegmattis* ATCC 14468, *Escherichia coli* NCTC 1066, *Vibrio cholera* (clinical isolate), and one fungus *Cryptococcus neoformans* (local clinical isolate). All Clinical isolates were obtained from the Department of Microbiolgy, Muhimbili University of Health and Allied Sciences (MUHAS). Minimum inhibitory concentrations were determined using micro-dilution techniques as described by Ellof (1998). The 96 well microtitre plates (Corning® Thermowell PCR 96 well plates, Sigma-Aldrich) were used for each yeast extract tested in duplicate at serial dilutions of 0.08, 0.16, 0.31, 0.625, 1.25, 2.5, 5 and 10 mg/ml. The first two columns were used for solvent controls while the last two for positive controls, Gentamycin Sulphate for bacteria and

Clotrimazole for fungus (0.024 – 50 µg/ml). In each well, 100µl of broth was added, followed by addition of 50µl of crude extracts (100 mg/ml). Thereafter 50µl was drawn from each well in the first row and added into the next row downwards in all columns. Then 50µl of broth inoculated with test organism was added and then the plates were incubated overnight at 37°C. Forty microlitres of 0.2mg/ml *p*-iodonitrotetrazolium chloride (NIT) dye were added into each well and incubated for another two hours. Plates were inspected for colour change; blue colour was an indication of microbial growth. The last wells in row in which there was no colour change were recorded as MICs.

2.6 Data analysis

Probity analysis was performed using SPSS 15.0 software which gives the regression equation. With regression equation LC_{50} were calculated at 95% confidence intervals. A LC_{50} greater than 100µg/ml was considered to represent a non toxic extract (Moshi et al., 2010). Proposed MIC was; up to 0.5 mg/ml was regarded as strong inhibitors, between 0.6 mg/ml to 1.5 mg/ml as moderate inhibitors and weak inhibitors when MIC is above 1.6 mg/ml (Aligiannis et al., 2001).

3. Results and Discussion

3.1 Extract yield

Results on the concentration of 18 yeasts crude extracts obtained from 14 day culture filtrates are presented in (Figure 1). Two yeast isolates from the *Galactomyces* group both originating from Kilimanjaro (Gg5Kili1, Gg6Kili2), one isolate from the *Pichia* group from Mwanza (PkMw1) and one isolate from the *Candida* group from Mwanza (Cp3Mw5) produced highest amounts of extract of 4290 µg/ml, 4100 µg/ml, 5100 µg/ml and 4500 µg/ml, respectively.

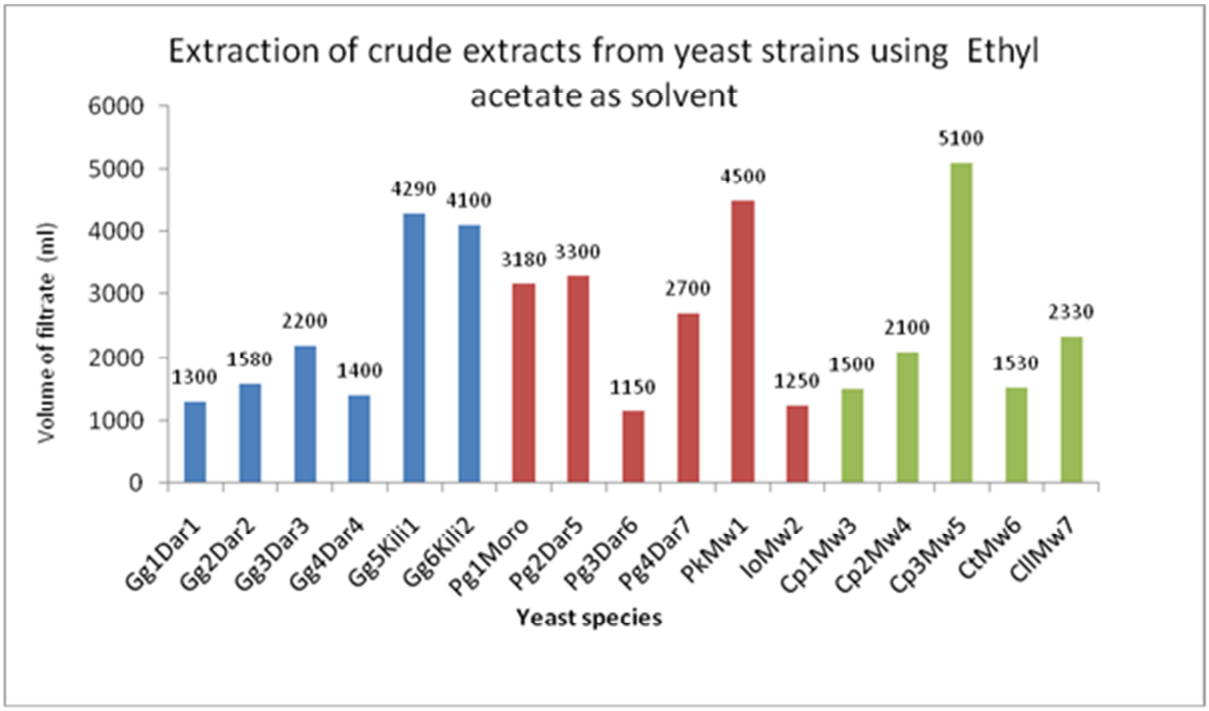

Figure 1: Amount of extract obtained from various volumes of 14 days culture filtrates, Origin of species: *Dar-Dar es Salaam Moro-Morogoro Mw- Mwanza Kili- Kilimanjaro*. Blue bars representing *G. geotrichum*, red bars representing *Pichia* and *I. orientalis* group and green colored bars are for a group of *Candida* and *Cl. Lusitaniae*.

3.2 Brine Shrimps Lethality Test

The brine shrimp lethality test results (Table 1) showed that LC_{50} of all 18 yeast extracts ranged from 87.9 µg/ml to over 1,000 µg/ml. Only two extracts from Cp3Mw5 (C. pararugosa) and Pg4Dar7 (G. geotrichum) showed

some toxicity by having LC_{50} values of 87.9 µg/ml and 99.0 µg/ml respectively. The remaining had LC50 values greater than 100 which is traditionally considered as non toxic (Moshi et al., 2010). Crude extract from isolate Gg6Kili2 exhibited by far the lowest cytotoxicity with LC_{50} of 1187.3µg/ml, followed by CtMw6 (LC_{50} 410.0 µg/ml) and Gg5Kili1 (LC_{50} *350.9 µg/ml) (Table 1).* According to Moshi *et al.* (2010), LC_{50} greater than 100 µg/ml is considered non toxic. Toxicity of extract of mangrove *P. guilliermondii* on human Celline had been reported by Joel and Bhimba, (2013). Moshi and others in (2010) suggested that LC_{50} below 100µg/ml indicates toxic extracts which may be associated with anti-cancer activity.

Table 1: Brine shrimp toxicity of Ethyl acetate extracts of yeast isolates from traditional fermented milk expressed as LC_{50} µg/ml (95% Confidence Interval)

Extract	Regression Equation	LC50 (µg/ml)	Regression coefficient (R^2)
Gg1Dar1	y = 0.0036x + 0.117	106.4	0.737
Gg2Dar2	y = 0.004x + 0.0469	113.3	0.8616
Gg3Dar3	y = 0.002x + 0.2876	106.2	0.282
Gg4Dar4	y = 0.0034x - 0.0504	132.2	0.9839
Gg5Kili1	y = 0.0014x + 0.0087	350.9	0.6797
Gg6Kili2	y = 0.0004x + 0.0251	1187.3	0.4692
Pg1Moro	y = 0.0037x + 0.0281	127.5	0.8785
Pg2Dar5	y = 0.0036x - 0.0907	113.7	0.9067
Pg3Dar6	y = 0.0035x + 0.0406	131.3	0.9102
Pg4Dar7	y = 0.0045x + 0.0547	99.0*	0.7481
PkMw1	y = 0.0033x + 0.028	143.0	0.8093
IoMw2	y = 0.0028x + 0.0376	165.1	0.8322
Cp1Mw3	y = 0.0028x + 0.0043	177.0	0.8904
Cp2Mw4	y = 0.0025x - 0.0014	199.4	0.7876
Cp3Mw5	y = 0.0027x + 0.2577	89.7*	0.4233
CtMw6	y = 0.0012x + 0.008	410.0	0.8301
ClMw7	y = 0.0027x - 0.0284	174.7	0.937
NDMw8	y = 0.0032x - 0.063	136.6	0.9489

* The toxic crude extracts on brine shrimp LC_{50} <100 µg/ml

3.3 Antimicrobial activity

Minimum inhibitory concentration analysis (Table 2) revealed promising activity against tested bacteria and fungus in 61% of all extracts (MIC ≤ 1.25 mg/ml). All extracts from *Candida* and *Cl. Lusitaniae,* all from Mwanza region demonstrated the highest activity against *C. neoformans* having MIC < 1mg/ml and moderate activity agaisnt *E. Coli* and *V. Cholera*. Same extracts together with extracts from one of *G. geotrichum* from Dar es Salaam and *P. guilliermondii* (Pg1Moro) from Morogoro exhibited promising activity against *Mycobacterium smegmattis* with MIC of 1.25 mg/ml each.

Extract of *C. tropicalis* CtMw6 had shown to exhibit the broadest antimicrobial activity against three bacteria *S. aureus, P. aureginosa* with MIC of 0.1563 mg/ml in both aspects and against *M. smegmattis* (MIC 1.25 mg/ml) and antifungal activity against *C. neoformans* (0.1563 mg/ml). The extract of isolate Pg1Moro was the only one to exhibit promising activity against *V. cholera* with MIC of 1.25 mg/ml. Moreover, the same extract from *P. gulliermondii* from Morogoro region (Pg1Moro) and also *I. Orientalis* (IoMw2) had shown promising activity against *E. coli* (MIC, 1.25 mg/ml). Of all Dar es Salaam samples, extracts from *G. geotrichum* (Gg1Dar1 and Gg2Dar2) TFMD1 and TFMD2 had promising bioactivity against *C. neoformans* and *M. smegmattis*, respectively (MIC, 1.25 mg/ml). Based on the argument by Alliagnis *et al.* (2001), an extract with MIC below 0.5 mg/ml is regarded as strong inhibitor.

Table 2 : Antimicrobial activity of crude extracts from yeasts isolated from traditional fermented milk expressed as minimum inhibitory concentration (MIC) (mg/ml)

Yeast isolate extract	Minimum Inhibitory Concentration (mg/ml)						
	C. neoformas	*E. coli*	*V. cholera*	*S. aureus*	*S. faecalis*	*P. aureginosa*	*M. smegmatis*
Gg1Dar1	5	-	2.5	5	2.5	5	1.25
Gg2Dar2	1.25	5	5	10	10	-	5
Gg3Dar3	5	5	5	10	5	-	5
Gg4Dar4	5	5	5	10	5	-	5
Gg5Kili1	-	5	5	5	5	-	5
Gg6Kili2	5	5	5	10	10	-	5
Pg1Moro	0.1563	-	1.25	-	2.5	-	1.25
Pg2Dar5	5	5	5	10	10	-	5
Pg3Dar6	5	5	5	10	10	-	5
Pg4Dar7	5	5	5	5	10	-	10
PkMw1	0.3125	-	2.5	5	2.5	2.5	1.25
IoMw2	0.1563	1.25	2.5	5	2.5	5	1.25
Cp1Mw3	0.625	2.5	2.5	2.5	5	5	1.25
Cp2Mw4	0.3125	5	2.5	5	2.5	2.5	1.25
Cp3Mw5	0.3125	1.25	2.5	5	2.5	2.5	1.25
CtMw6	0.1563	-	-	0.1563	5	0.156	1.25
CllMw7	0.3125	-	2.5	5	2.5	2.5	1.25
NDMw8	0.625	-	2.5	2.5	5	5	1.25
Gentamycin	NA	0.003125	0.001563	0.000781	0.0000244	0.001563	NA

In this study, majority of the crude extracts isolated from Mwanza fermented milk exhibited strong inhibition against *C. neoformas* and only one of the extracts against *S. aureus* and *P. aurigenosa*. Moderate activity against *E. coli* was exhibited by *C. pararugosa* (Cp5Mw5, MIC = 1.25). It is worth noting that in this research the same extracts with strong antifungal activities also exhibited considerable antibacterial activity (1.25 mg/ml) against *M. smegmattis,* a Tuberculosis (TB) causing organism. Antimicrobial activity of fermented milk is reported to be attributed to the microbial production of bioactive metabolites such as certain vitamins, bioactive peptides, organic acids or fatty acids during fermentation (Stanton *et al*., 2005). Indeed, a report by Mufandaedza *et al*. (2006) that *Escherichia coli* were significantly reduced in the traditionally fermented milk containing yeasts and bacteria from Zimbabwe is in support of our findings.

Studies elsewhere have reported presence of antimicrobial agents in fermented milk which may play an important therapeutic role to consumers. A study by Yesillik *et al*., (2011) reported that homemade yoghurt inhibited the growth of *P. aureginosa* as compared to commercial yoghurt. Similarly, crude extracts of secondary metabolites of some yeasts such as *P. guilliermondii* have been reported to exhibit promising antimicrobial activity against various pathogenic microorganisms (Joel and Bhimba, 2013; Lahlali etal., 2011; Zhao *et al*., 2010). It was reported in Russia that at the time post World War II, in areas where no medical facilities were available, kefir, fermented milk product fermented by co-starter culture comprising of Lactic acid bacteria (LAB) and yeasts was administered to TB patients (Lopitz and Aitor, 2006; Cevikbas et al., 1994). Mariam (2009) , reported that, filtrate from Ethiopian traditional fermented milk enriched with a mixture of Lactic acid bacteria (LAB) strains inhibited spiked M. bovis from the mixture in 14 day. Moreover, industrially fermented milk enriched with yeasts (kefir and koumiss) were claimed to treat gastrointestinal disorders when modern treatments was not available (Stanton et al., 2005).

Yeasts found in several samples of African indigenous fermented foods and beverages are being related to improvement of nutritional value, pro-biotic effects and inhibition of undesired microorganisms (Paucean *et al*., 2010, Jespersen, 2003; Stanton *et al*., 2005). It has also been reported that yoghourt enriched with yeasts produce bio-therapeutic agents effective in treatment of diarrhoea in children and critically ill tube fed patients (Lim &

Tay, 2011;Yesillik et al., 2011; Dixit and Gandhi, 2006). Results from this study echoes the important role played by microorganisms found in fermented milk. Generally our results revealed that extracts from the studied yeast strains have high potential to be used as bio-therapeutic agents since more than 80% had either or/and strong to weak activity against all tested pathogens and were found to be non- toxic.

4. Conclusion

In this study, crude extract from yeasts isolated from Tanzanian 'mtindi' which was made through spontaneous fermentation of raw milk were found to be effective against some of the bacterial and fungal strains studied. It may be suggested from these findings that these extracts can be a potential source of natural antimicrobial compounds. The identification of the bio-active group of the extracts will provide the basis for further research on the actual active compounds' names and chemical structures.

5. Acknowledgement

This research was supported by the SIDA-SAREC and Muhimbili University of Health and Allied Sciences (MUHAS). We are grateful to Dr. Sung'wa of Chemistry Department, University of Dar es Salaam for his technical assistance during extraction of the crude extracts. We are also thankful to Dr. E. Innocent, the Director and the then Head of the Natural products department, Muhimbili University of Health and Allied Sciences for her support in the Bioassays and lastly but not least to Mr. A. Kidukuli for his technical support.

6. References

Akabanda, F., & Glover, R. L. K. (2010). Microbiological Characteristics of Ghanaian Traditional Fermented Milk Product , Nunu. *Nature and Science*, *8*(9), 178–187.

Antibacterial Effects of Some Fermented Commercial and Homemade Dairy Products and Lactic Acid against Selected Foodborne Pathogens. (n.d.).

Cevikbas, A., Yemni, E., Ezzedenn, F. W., Yardimici, T., Cevikbas, U., & Stohs, S. J. (1994). Antitumoural antibacterial and antifungal activities of kefir and kefir grain. *Phytotherapy Research*, *8*(2), 78–82. doi:10.1002/ptr.2650080205

Chowdhury, N., Ghosh, A., & Chandra, G. (2008). Mosquito larvicidal activities of Solanum villosum berry extract against the dengue vector Stegomyia aegypti. *BMC Complementary and Alternative Medicine*, *8*, 10. doi:10.1186/1472-6882-8-10

Dixit, K., Gandhi, D. N. (2006). Biotherapeutic properties of probiotic yeast Saccharomyces species in fermented dairy foods. *[On-Line] UK*.

Gran, H. M., Gadaga, H. T., & Narvhus, J. A. (2003). Utilization of various starter cultures in the production of Amasi, a Zimbabwean naturally fermented raw milk product. *International Journal of Food Microbiology*, *88*(1), 19–28. doi:10.1016/S0168-1605(03)00078-3

Isono, Y., & Shjngu, I. (1994). and Characteristics of Lactic Acid Bacteria Isolated from Masai Fermented Milk in Northern Tanzania. *Biosc. Biotech. Biochem.*, *58*(4), 660–664.

Jespersen, L. (2003). Occurrence and taxonomic characteristics of strains of Saccharomyces cerevisiae predominant in African indigenous fermented foods and beverages. *FEMS Yeast Research*, *3*, 191–200. doi:10.1016/S1567-1356(02)00185-X

Joel, E. L., & Bhimba, B. V. (2013). Evaluation of secondary metabolites from mangrove associated fungi Meyerozyma guilliermondii, 189–194.

Kavas, G., Kinik, H., Uysal, S., Kilic, N., A. (2006). Characteristics of Yeasts Isolated from Artisan Turkish Dairy Products.pdf. *International Journal of Dairy Sciences*, *1*(1), 44–50.

Kuwabara, Y., Nagai, S., Yoshimitsu, N., Nakagawa, I., Watanabe, Y., & Tamai, Y. (1995). Antihypertensive effect of the milk fermented by culturing with various lactic acid bacteria and a yeast. *Journal of Fermentation and Bioengineering*, *80*(3), 294–295.

Lahlali, R., Hamadi, Y., Guilli, M. El, & Jijakli, M. H. (2011). Efficacy assessment of Pichia guilliermondii strain Z1, a new biocontrol agent, against citrus blue mould in Morocco under the influence of temperature and relative humidity. *Biological Control*, *56*(3), 217–224. doi:10.1016/j.biocontrol.2010.12.001

Lim, S. L., & Tay, S. T. (2011). Diversity and killer activity of yeasts in Malaysian fermented food samples. *Tropical Biomedicine*, *28*(2), 438–43. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22041766

Lourens-Hattingh, A., & Viljoen, B. C. (2002). Survival of dairy-associated yeasts in yoghurt and yoghurt-related products. *Food Microbiology*, *19*(6), 597–604. doi:10.1006/fmic.2002.0515

Mariam, S. H. (2009). Interaction between lactic acid bacteria and Mycobacterium bovis in Ethiopian fermented milk: insight into the fate of M. bovis. *Applied and Environmental Microbiology*, *75*(6), 1790–2. doi:10.1128/AEM.01943-08

Mlimbila, J. S., Hosea, K. M., & Muruke, M. (2013). Molecular identification and proteinase activity of yeasts isolated from fermented milk, *2*(2), 7–14.

Moshi, M. J., Innocent, E., Otieno, J. N., Magadula, J. J., Otieno, D. F., & Mbabazi, P. (2010). Antimicrobial and brine shrimp activity of Acanthus pubescens root extracts. *Tanzania Journal of Health Research*, *12*(2).

Mufandaedza, J., Viljoen, B. C., Feresu, S. B., & Gadaga, T. H. (2006). Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic Escherichia coli and Salmonella enteritidis strains. *International Journal of Food Microbiology*, *108*(1), 147–52. doi:10.1016/j.ijfoodmicro.2005.11.005

Narvhus, J., & Narvhus JA, G. T. (2003). The role of interaction between yeasts and lactic acid bacteria in African fermented milks: a review. *International Dairy Journal*, *11*(1-2), 63–70. doi:10.1016/S0168-1605(03)00247-2

Ni, H. J., Bao, Q. H., Sun, T. S., Chen, X., & Zhang, H. P. (2007). [Identification and biodiversity of yeasts isolated from Koumiss in Xinjiang of China]. *Wei Sheng Wu Xue Bao= Acta Microbiologica Sinica*, *47*(4), 578–582.

Otsoa Fernando Lopitz, Rementeria Aitor, E. N. G. J. (2006). Kefir: A smbiotic yeasts-bacteria community with alleged healthy capabilities. *Rev Iberoam Micol*, *23*, 67–74.

Paucean, A., Socaciu, C., Vodnar, D. A. N., & Elena Mudura. (2010). A new functional dairy product containing an optimized mixture of Lactococcus bacteria , kefir and brewer ' s yeasts. *Romanian Biotechnological Letters*, *15*(6), 5793–5800.

Samet-bali, O., Ennouri, M., Dhouib, A., & Attia, H. (2012). Characterisation of typical Tunisian fermented milk: Leben. *African Journal of Microbiology Research*, *6*(9), 2169–2175. doi:10.5897/AJMR12.065

Savova, I., & Nikolova, M. (2002). ISOLATION AND TAXONOMIC STUDY OF YEAST STRAINS FROM BULGARIAN DAIRY PRODUCTS. *Journal of Culture Collections*, *3*(2000-2002), 59–65.

Stanton, C., Ross, R. P., Fitzgerald, G. F., Sinderen, D. Van, & Van Sinderen, D. (2005). Fermented functional foods based on probiotics and their biogenic metabolites. *Current Opinion in Biotechnology*, *16*(2), 198–203. doi:10.1016/j.copbio.2005.02.008

Zhao, J., Mou, Y., Shan, T., Li, Y., Zhou, L., Wang, M., & Wang, J. (2010). Antimicrobial metabolites from the endophytic fungus Pichia guilliermondii isolated from Paris polyphylla var. yunnanensis. *Molecules (Basel, Switzerland)*, *15*(11), 7961–70. doi:10.3390/molecules15117961

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar