ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info/jasr

ISSN
0976-9595
Research Article

BIOLOGICAL POTENTIALS OF EXTRACTS AND COMPOUNDS FROM *MAMMEA USAMBARENSIS* VERDC FRUIT

Pax J. Masimba[1*], Joseph J. Magadula[1], Zaituni Msengwa[1], Rose B. Tarimo[1], Zakariah H. Mbwambo[1], Matthias Heydenreich[2], Dimitri Breard[3], Pascal Richomme[3], Eltayeb Fadul Fadul Alla[4]

[1]*Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania.*

[2]*Institut fur Chemie, Universitat Potsdam, Postfach 601553, D-14415 Potsdam, Germany.*

[3]*SONAS-UFR Sciences Pharmaceutiques, 16 Bd Daviers, F-49100 Angers, France*

[4]*National Center for Research, P. O. Box 2404, Khartoum, Sudan*

**Corresponding author: paxmasimba@gmail.com*

ABSTRACT

In attempt to investigate some biological activities of *Mammea usambarensis* fruit, absolute ethanolic crude extract, 80% DCM/PE fraction, 5% MeOH/DCM fraction and mammea B/AB were subjected to toxicity test in brine shrimps and mice, antimicrobial activity by disc diffusion and broth dilution method and antioxidant activity by DPPH scavenging activity.

In the brine shrimps toxicity test, the crude extract, 80% DCM/PE fraction, 5% MeOH/DCM fraction and mammea B/AB were highly toxic with LC_{50} values of 1.58, 1.14, 3.89 and 5.21 µg/ml, respectively. **T**he crude extract was non-toxic to mice even at a dose above 2000 mg/kg body weight. The crude extract, 80% DCM/PE 5% MeOH/DCM fractions and mammea B/AB exhibited low activity with zone of inhibition of 8.3-16 mm for *Salmonella kisarawe,* 8.7-14 mm for *Staphylococcus aureus* and 8.3-10 mm for *Klebsiella oxytoca.* The minimum inhibitory concentrations ranged from 3.75 µg/µl-25 µg/µl. All samples tested had no activity against *Candida albicans* and *Cryptococcus neoformans*. In DPPH assay crude extract, 80% DCM/PE fraction, 5% MeOH/DCM fraction and mammea B/AB had lower scavenging activity of 86, 69, 72 and 77 µg/ml: mM, respectively, compared to the standard compound, propagyl gallate, which had a value of 93 µg/mL: mM. To conclude, the antioxidant activity exhibited prompt further studies aimed at discovery of antioxidant agents.

Keywords: *Mammea usambarensis* fruit, toxicity, anti-microbial activity, anti-oxidant activity

1. INTRODUCTION

Plants have been used as a source of medicine since ancient times for treating diseases in human and animals [1]. The ethno medicine information of plants provide a clue for identification of compounds for combating ill health conditions. This has attracted the attention of researchers to investigate and predict the safety of plants and also to search for possible alternatives to fight the existing incurable diseases as well as the problem of microbes developing drug resistance [2].

Even though there are several documentations regarding traditional uses of some common plants used as herbal medicine, yet there are few records on their toxicity and efficacy. Toxicological studies help to decide whether a new drug should be subjected to clinical use or not [3, 4]. Therefore it is necessary to evaluate the safety and efficacy of these plants before formulation into useful products.

Worldwide there is a growing problem of drug resistance and degenerative diseases necessitating a need to search for new therapeutic agents [5, 6]. Recently there has been an increased interest in searching for natural antimicrobial and antioxidants present in medicinal plants [7, 8]. Plants are a good source of antimicrobials and antioxidants [9, 10]. Previously, the crude and fractions from roots and stems of *M. usambarensis* were shown to be effective as antimicrobials and antioxidants [10, 11]. This study aimed to investigate the toxicity, antimicrobial and antioxidant activities of the fruit part of *M. usambarensis*. Once found effective and safe, the fruits can be formulated into herbal drugs instead of roots and stem part which threatens the life of the plant.

2. MATERIALS AND METHODS

2.1. Reagents

Ethanol (absolute) was purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands), Dimethyl sulfoxide (DMSO) was from Sigma® (Poole, Dorset, UK), Tryptone Soya agar and broth from HIMEDIA® (Himedia Laboratories Pvt Ltd, Mumbai, INDIA), *p*-Iodonitrotetrazolium chloride was bought from SIGMA® (Sigma- Aldrich®, St Louis, USA), 96 well microtitre plates were supplied by KAS medics Tanzania, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (L'Isle d'Abeau Chesnes, France) while sea salt was prepared

locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast.

2.2. Ethical Consideration

During the study the following issues were taken into consideration: Few mice were kept in each cage to enable mice to express their normal behavior. Clean water and appropriate feed was given and mice were kept at the temperature of 22°C (±3°C) and the relative humidity was 50-60%. To reduce pain prior to dissection of the mice, chloroform/diethyl ether was used to induce anesthesia and death (euthanasia). An ethical clearance was given by the Director of Research and Publications of Muhimbili University of Health and Allied Sciences (MUHAS).

2.3. Microorganisms

Authentic pure cultures of human pathogenic bacteria: *Staphylococcus aureus* (NCTC 25923), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (NCTC 8385), *Salmonella kisarawe* (Clinical isolates), *Klebsiella oxytoca* (ATCC 13182), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhimurium* (clinical isolates), *Salmonella typhi* (NCTC 8385) and two fungi species, *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (clinical isolate) were obtained from the Department of Microbiology, Muhimbili University of Health and Allied Sciences (MUHAS). The Brine Shrimps eggs were purchased from Aquaculture innovations (Grahamtown 6140, South Africa).

2.4. Plant Collection and Identification

The fruits of *M. usambarensis* were collected from Shugayu forest reserve, Lushoto district, Tanga region with the help of a field botanist, Mr Haji Suleiman, from the Botany department, University of Dar-es-salaam, Tanzania. The herbarium specimen were kept in the botany department of the University of Dar-es-salaam, Tanzania with code number HOS 3502.

2.5. Extraction of Plant Materials

The fruits were prepared in the field by chopping and then air dried. The dried materials were milled into a pulverized powder using a milling machine available at the Institute.

The method of percolation was employed during the extraction process. Pulverized fruits of the plant were soaked in 100% ethanol at room temperature (25-33°C) for 24 hours and then filtered. The filtrate was concentrated in a rotary evaporator to remove solvent. The crude extract obtained was placed in the freezer for few hours and then subjected to freeze drier to remove any solvent that could have remained.

2.6. Isolation of Tested Compound and Fractions

The crude extract of the fruit of *M. usambarensis* (30 g) was packed to Column chromatography over silica gel to give 99 fractions of 10 ml each. Starting with eluting with solvent with low polarity 100% PE followed by increasing the polarity to 100% DCM and later to 10% MeOH. The combined fraction 1-11 (20%) DCM/PE showed only long chain fatty acids as analyzed by TLC, so were not followed up. Combined fraction 12-35 were eluted by 50% DCM/PE, on the TLC two compounds were observed. The two compounds were rechromatographed over Silica gel eluted with (50% DCM/PE) then by preparative over silica gel to obtain mammea B/AB (26.4 mg). Furthermore the combined fraction 36-43 (80% DCM/PE) contained mammea B/AB. Another combined fraction 44-63 eluted by 85% DCM/PE was then purified by repeated crystallization in methanol and observed as pure compound on the TLC and labeled as mammea B/AB (1.4 g). Combined fractions 64-99 (5% MeOH/DCM) as indicated on the TLC the presence of sugar compounds and therefore were not followed. Therefore the combined semi purified fractions of 36-43 (80% DCM/PE) and 64-99 (5% MeOH/DCM), mammea B/AB and crude extract were used in bioassays because their amounts were enough. Only the crude extract was subjected to acute toxicity test in mice.

2.7. Toxicity Testing

2.7.1. Brine Shrimp Test

The experiment was set according to the method of Meyer and others [12]. Briefly, 3.8 gm of artificial sea salt was dissolved in 1 litre of distilled water and 2 g of brine shrimp eggs were added and left for 24 hours to hatch in light condition. Extracts were dissolved in DMSO and tested at concentrations of 240, 120, 80, 40, 24, 8, 4.5, 3, 1.5, 1.0, 0.5 and 0.3 μg/ml using ten brine shrimps larvae. Each level of concentration was tested in duplicate. The negative control contained brine shrimp, artificial sea water and DMSO (0.6%) only. The vials were incubated for 24 hours and mean percentage mortality rates were calculated. The graph of mean percentage mortality against log concentration were drawn and subjected to analysis using Excel computer program so as to get the concentration required to kill 50% of larvae (LC_{50}) and confidence interval.

2.7.2. Acute Oral Toxicity

The OECD (Organization of Economic Co-operation and Development) guideline number 423 of 2001 for testing of chemicals was used to test Acute Oral Toxicity test in mice by Acute Toxic Class Method [13]. Albino mice of both sex weighing 17-24 g from the animal house of the Muhimblili University of Health and Allied Sciences were acclimatized for 5 days before the treatments. Standard diet and water were provided. Twelve albino mice were randomly selected and divided into two groups of six mice. The mice were fasted four hours before administration of the extract. The extract was dissolved in water. Then one group was given water solvent as controls and the treated groups were given doses of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight of the extract according to the guideline [13]. Food was withheld for 1-2 hours after administration of the extract but not water. The mice were observed regularly for any sign of toxicity and death during the first 24 hours and daily for 14 days. At the end

of study all mice involved in the study period were weighed, euthanized and dissected for macroscopic organ analysis. The liver, spleen, kidney, lungs and heart were removed, blotted free of blood and weighed immediately.

2.8. Antimicrobial Assay

The disc diffusion assays for bacteria were carried out in tryptone soya agar while that of fungi was carried in Sabouraund's dextrose agar. Solvent discs were used as the negative controls while standard gentamycin (10 µg/disc) and clotrimazole (20 µg/disc) were used as the positive controls for bacteria and fungi respectively. The paper disks were each impregnated with 10 µl (500 mg/ml for crude extract and fractions and 5 mg/ml for mammea B/AB) of test samples. Two fold microdilution assay [14, 15] was used to determine MIC for the test materials.

2.9. Antioxidant Assay

The DPPH radical scavenging was determined according to the method of Shimada *et al.,* 1992 [16]. In 96-wells plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2.10. Data Analysis

The BST assay was analyzed by using excel programme. The graph of percentage mortality rate was drawn against log concentration to obtain equation of a line which was used to calculate the LC_{50}.

In Acute Oral Toxicity, the Student's *t*-test (SPSS computer program) was employed to analyze the results statistically so as to compare mean relative organ weight between the tested and control groups. All values were expressed as mean ± S.D differences and whenever the p-value was below the probability level of 0.05, this was considered statistically significant.

Antioxidant results were presented as means±SEM (standard error of the means). Statistical analysis for all the assays results was done using Microsoft Excel program. The IC_{50} values were determined by EZ-Fit Program (Perrella Scientific Inc. Amherst, U. S. A).

3. RESULTS AND DISCUSSION

3.1. Brine Shrimp Lethality Test

The brine shrimp test is used as a preliminary test for testing toxicity of a plants after a single dose administration. The brine shrimp results are interpreted as follows: 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 LC_{50} > 100 µg/ml as non-toxic [17]. It is also used to predict for anticancer activity of an extract. The study done by Moshi *et al.,* 2004; 2006 provided the evidence that plant extract with the LC_{50} less than 20 µg/ml could be a source for anticancer compounds [18, 19]. The crude extract, semi purified fractions and pure compound had the LC_{50} of 1.58 µg/ml, 1.14 µg/ml, 3.89 µg/ml and 5.21 µg/ml respectively (Table 1). All the tested samples were therefore toxic to brine shrimp larvae and had the LC_{50} less than that of a standard anticancer drug, cyclophosphamide which has LC_{50} 16.37 µg/ml, suggesting that the samples could be potential anti-cancer agents provided they will exhibit selective toxicity to cancerous cells.

Table 1: Results of Brine Shrimps Test of M. Usambarensis Fruit

Sample name	LC_{50} (µg/ml)	95% Confidence Intreval
1	1.58	(1.33, 1.87)
2	1.14	(0.89, 1.46)
3	5.21	(4.09, 6.62)
4	3.89	(2.96, 5.10)

***Key**: 1= crude extract, 2= 80% DCM/PE fraction, 3= mammea B/AB and 4= 5% MeOH/DCM fraction*

3.2. Acute Toxicity Test in Mice

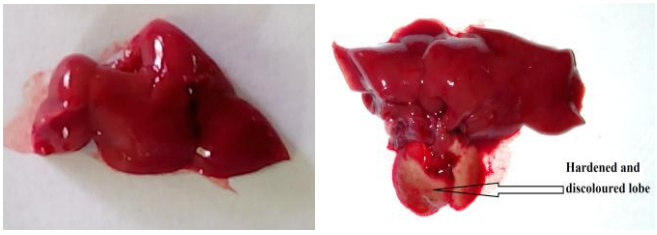
It determines the range of doses that are toxic to organisms. After oral administration of the crude extract of *M. usambarensis*, some parameters were observed for the first 24 hours then daily for 14 days. The following parameters were observed: change in back hair, excitement, breathing difficulty, diarrhea, sleep/comma, eyelid closure, death, body weight, relative organ weight and organ morphological changes. There were no signs of toxicity displayed by mice at all doses except at 2000 mg/kg body weight in which one mouse showed liver abnormality in the pathological examination.

The results (Table 2) showed that there was increase in body weight in day 14 for both control and treated mice groups. Furthermore, the mean differences in body weight between day 0 and day 14 were statistically significant at all doses implying that the extract did not interfere with the normal growth of the mice.

At day 14 all mice were dissected to examine their internal organs. The observed organs were all normal except for one mouse at dose 2000 mg/kg body weight. This mouse had abnormal liver whereby the small lobes seemed to be hardened and had discoloration that suggested the signs of liver cirrhosis (Figure 1b). Since only one mouse displayed this sign of toxicity it cannot be concluded that this was due to the effect of the extract. It is possible that the mouse had the problem before the beginning of the experiment. To rule out this possibility some more experiments are needed.

Table 2: Effect of M. Usambarensis Fruit Crude Extract in Body Weight of Mice

Group	Dose	Mean (S.D) at Day zero	Mean (S.D) at Day 14	Mean difference	p-value
1	Control	19.515 (1.500)	23.473 (1.658)	3.958	0.000014
	5mg/kg bwt	19.303 (1.816)	23.528 (2.454)	4.225	0.000000
2	Control	19.515 (1.500)	23.708 (1.457)	4.193	0.000000
	50 mg/kg bwt	19.178 (1.894)	22.618 (2.285)	3.44	0.000000
3	Control	19.481 (1.470)	23.023 (2.037)	3.542	0.000000
	300 mg/kg bwt	19.319 (1.483)	22.442 (2.152)	3.123	0.000000
4	Control	19.515 (1.500)	23.390 (2.824)	3.875	0.000000
	2000 mg/kg bwt	19.265 (1.563)	21.994 (2.454)	2.729	0.000004



(a) (b)

Figure 1: Showing (a) Normal mouse liver (b) affected mouse liver

Despite that, the study done by Du *et al.,* 2010 reported the effect of phytochemicals of mammea-type coumarins in liver damage and neurogeneration and therefore the results from our study may partially be in agreement with this report [20]. As indicated in Table 3, the effect of the crude extract of *M. usambarensis* fruits on organ to body weight ratio in mice was similar between the treated and control groups, suggesting that the extracts did not interfere with the normal growth of the organs.

Table 3: Effect of Fruits Crude Extract of M. Usambarensis on Relative Organ Weight in Mice

Organ	Dose	Control Mean (S.D)	Treated Mean (S.D)	Mean difference	p-value
Spleen	5 mg/kg bwt	0.014690(0.0034)	0.013769(0.0018)	0.000921	1.000
	50 mg/kg bwt	0.015202 (0.0051)	0.013665 (0.0019)	0.001537	0.981255
	300 mg/kg bwt	0.015552(0.0047)	0.014233(0.0035)	0.001319	1.000
	2000 mg/kg bwt	0.015216(0.004)	0.015089(0.0023)	0.000127	1.000
Kidneys	5 mg/kg bwt	0.004865(0.0018)	0.004397(0.0012)	0.000468	0.905996
	50 mg/kg wt	0.00473(0.0019)	0.003538(0.001)	0.001192	0.990127
	300 mg/kg bwt	0.004845(0.0019)	0.004706(0.0024)	0.000139	1.000
	2000 mg/kg bwt	0.004801(0.0019)	0.004898(0.0022)	0.000097	0.910114
Liver	5 mg/kg bwt	0.058062(0.005)	0.055273(0.0064)	0.002789	0.999915
	50 mg/kg bwt	0.057372(0.006)	0.05592(0.0078)	0.001452	0.986712
	300 mg/kg bwt	0.060417(0.0058)	0.053336(0.0077)	0.007081	0.99987
	2000 mg/kg bwt	0.057935(0.0074)	0.058564(0.0098)	0.00063	0.729428
Lungs	5 mg/kg bwt	0.00488(0.001)	0.004328(0.0004)	0.000552	0.999915
	50 mg/kg bwt	0.004537(0.0005)	0.004509(0.0006)	0.000028	0.986712
	300 mg/kg bwt	0.004509 (0.0006)	0.004473(0.0004)	0.000036	0.99987
	2000 mg/kg bwt	0.004565 (0.0003)	0.004277(0.0005)	0.000288	0.729428
Heart	5 mg/kg bwt	0.007865(0.0017)	0.006088(0.0011)	0.00178	0.99988
	50 mg/kg bwt	0.00788(0.0016)	0.00707(0.0014)	0.000809	0.999707
	300 mg/kg bwt	0.008162(0.0018)	0.007727(0.0012)	0.000435	0.999511
	2000 mg/kg bwt	0.008086(0.0019)	0.007387(0.0014)	0.000699	0.999455

Previous studies reported the effects of prenylated mammea-type coumarins on the mitochondria. These types of compounds are weak acidic in nature so they disrupt the mitochondrial function [20]. However, the result from this study calls more investigation on the possibility of *M. usambarensis* fruits to display mitochondria interruption. Since only one mouse exhibited a sign of toxicity even after pathological examination at all doses, the LD_{50} for the plant crude extract was considered to be ≥2000 mg/kg body weight [21]. Hence, basing on the observed parameters the fruit extract of *M. usambarensis* is considered to be non toxic to mice.

3.3. Antimicrobial Test Results

The antimicrobial sensitivity results obtained in this study (Tables 4 and 5) showed that crude extract, mammea B/AB and semi purified fractions were active against *Salmonella kisarawe*, *Staphyloccocus aureus* and *Klebsiella oxytoca*. Mammea B/AB and a fraction eluted from 80% DCM/PE were more active with inhibition zones ranging from 9 to 16 mm. The crude extract and 5% MeOH/DCM fraction on the other hand had inhibition zones ranging from 8 to 10 mm. All the samples tested were inactive against *Proteus sp*, *Klebsiella pneumoniae, Salmonella typhimurium, Salmonella typhi, Eschelichia coli*, *Candida albicans* and *Cryptococcus neoformans*.

Table 4: Antimicrobial Results of M. usambarensis Fruit by Disc Diffusion Method

Microorganism	Zone of inhibition (mm)					
	1	2	3	4	Gentamycin	Clotrimazole
Salmonella kisarawe	9.7±0.6	13.3±0.12	16±0.1	8.3±0.06	20	N/A
Staphylococcus aureus	9.0±0.00	12.3±0.12	14±0.00	8.7±0.06	18	N/A
Klebsiella oxytoca	9.3±0.12	9.3±0.23	10.0±0	8.3±0.06	13	N/A
Proteus species	-	-	-	-	10	N/A
Klebsiella pneumoniae	-	-	-	-	10	N/A
Salmonella typhimurium	-	-	-	-	17	N/A
Salmonella typhi	-	-	-	-	19	N/A
Eschelichia coli	-	-	-	-	14	N/A
Candida albicans	-	-	-	-	N/A	18
Cryptococcus neoformans	-	-	-	-	N/A	20

Key: *The values presented as mean inhibition zone (mm) ± S.D of three replicates with disc diameter inclusive (5mm), - = Not active, N/A= not applicable, 1= Crude extract, 2= 80% DCM/PE fraction, 3= Mammea B/AB and 4= 5% MeOH/DCM fraction*

As shown in Table 5, the fraction of 80% DCM/PE was more active than other fractions against *K. oxytoca*. According to Sartoretto *et al*, 2004, the MIC for potential plants are classified as follows; 0.05 -0.5 mg/ml (strong activity), 0.6-1.5 mg/ml (moderate activity) and MIC >1.5 mg/ml (weak activity) [22]. Therefore in the present study, since all MIC values were above 1.5 mg/ml, the tested extracts exhibited only weak activity (3.75 mg/ml-25mg/ml) for the sensitive bacteria strains tested.

Table 5: Minimum Inhibitory Concentration Results of M. Usambarensis Fruit

Microorganism	Minimum inhibitory concentration (mg/ml)				
	1	2	3	4	Gentamycin
Salmonera kisarawe	25	12.5	12.5	12.5	0.012
Klebsiela oxytoca	12.5	3.75	25	12.5	0.012
Staphyloccocus aureus	12.5	25	25	12.5	0.012

Key: *1= Crude extract, 2=80% DCM/PE fraction, 3= Mammea B/AB and 4= 5% MeOH/DCM fraction*

The unpublished work on the root and stem bark of *M. usambarensis* showed that some fractions were highly active against microbes [11]. This is contrary to our finding in which the fruits exhibited weak antimicrobial activity. Previously it has been shown that although plants are important source for the development of new chemotherapeutics agents, their pharmacological activities may vary with species and parts of the plants used [9]. Therefore our finding that the fruit exhibited lower antimicrobial activity than the root and stem bark of the same plant is not unusual.

3.4. DPPH Antioxidant Assay

The DPPH assay is used as a pre-screening method for investigation of new antioxidants from natural sources. DPPH has an absorption band at 517 nm which dissapear upon reduction by an antiradical compound or extract and this is reflected by the discolouration of DPPH solution [23]. The results from this study showed that the ethanolic crude extract, mammea B/AB, 80% DCM/PE and 5% MeOH/DCM fractions possess antioxidant activity that is lower than the standard (Propyl gallate) (Table 6). The crude extract however, had activity which was closer to the standard anti-oxidant agent used suggesting that it could be a potential source of antioxidant compounds.

Table 6: The DPPH Radical Scavenging Activities of M. usambarensis Fruit

S/N	Solvent	%RSA±SD (DPPH)
1	Ethanol	86±0.02
2	Dichloromethane	69±0.07
3	Dichloromethane	77±0.07
4	Methanol	72±0.02
PG	-	93±0.02

Key: *PG=Propyl gallate, 1= crude extract, 2= 80% DCM/PE fraction, 3= mammea B/AB and 4= 5% MeOH/DCM fraction*

Our results differ from the previous work done by Magadula, 2012, that reported a higher activity of the ethanolic crude extract of the stem bark of *M. usambarensis* which was twice than its standard drug, chlorogenic acid [10]. The two studies explored the differences in activity between two different parts of the plant (stem bark against fruits). Hence the stem bark of *M. usambarensis* stands a better chance of development into antioxidant agents if proven to be safe.

In conclusion, the investigation of safety explored toxicity effect of the fruits crude extract of *M. usambarensis* in brine shrimps and mice. The crude extract was highly toxic in brine shrimps and non toxic to mice. In additional to that, the semi purified fractions and pure compound of the fruit were also toxic to brine shrimp larvae giving a room for further studies aimed at the discovery of the anticancer compounds. Therefore studies on histopathological and biochemical profile of crude extract and compounds from *M. usambarensis* fruits are recommended. Even though the extracts from fruits did not exhibit high antimicrobial activity, the active antioxidant extracts could still serve as lead antioxidant molecules towards discovery of antioxidant agents. Therefore it is recommended that further studies be done on the fruit extracts that could lead into formulation of antioxidant herbal products.

4. ACKNOWLEDGEMENTS

The authors wish to thank Mr. Haji O. Suleiman of the Botany Department, University of Dar es Salaam, Tanzania for collection and identification of the plant materials.

5. REFERENCES

1. Mishra D. *Ind. J. Trad. Knowl*. 2013; **10:** 62-65.
2. Saga T. and Yamaguchi K. *Japanese Medical Association*, 2009; **52:** 103-108.
3. Aniagu SO, Nwinyi FC, Akumka DD, Ajoku GA, et al. *Afr. J. Biotech*, 2004; **4(1):** 72-78.
4. McNamara BP. Concepts in health evaluation of commercial and industrial chemicals In: Mehlman MA, Shapiro RE, Blumental H (Eds). New concepts in safety evaluation. Hemisphere. Washington DC., 1976;**1**: 61-140.
5. Kumar RS, Rajkapoor B, Perumal P. *Pharmacology line*, 2011; **1:** 710-720.
6. NIAID. *NIH Publication*, 2009; 3-9.
7. Preethi R, Deavanathan V, Loganathan M. *Adv. Biol. Res.,* 2010; **4(2):** 122-125
8. Silva BA, Ferreres F, Malva JO, Dias ACP. *Food Chem.,* 2005; **90:** 157-167.
9. Mahesh B. and Satish S. *World J. Agric. Sc.,* 2008; **4:** 839-843.
10. Magadula JJ. *Intern. J. Res. Phytochem. Pharmacol*. , 2012; **2(1):** 16-20.
11. Tarimo BR. *Un published report*, 2013.
12. Meyer BN, Ferrign RN, Putnam JE, Jacobson LB, et al. *Planta Med*., 1982; **45:** 31-34.
13. OECD. Acute Oral Toxicity- Acute Toxic Class Method. In: OECD Guideline for Testing of Chemicals, No Organization for Economic Cooperation and Development, 423; 2001.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. *Am. Clin. Pathol.,* 1966; **45(4):**, 493-496.
15. Luqman S, Srwastawa S, Darokar M P. *Pharm. Biol.,* 2005; **8:** 732-736.
16. Shimada K, Fujikawa K, Yahara K, Nakamura T. *J. Agric. Food Chem*., 1992; **40:** 945-948.
17. Moshi M, Otieno D, Weisheit A. *J. Ethnobot. Ethnomed.,* 2012; **1:** 8-14.
18. Moshi MJ, Cosam JC, Mbwambo ZH, Kapingu M, Nkunya MHH. *Pharmac. Biol.* , 2004; **42:** 547-551.
19. Moshi MJ, Mbwambo ZH, Nondo RSO, Masimba PJ, et al. *Afr. J. Trad., Compl. Altern. Medic*. , 2006; **3:** 48-58.
20. Du L, Mahdi F, Jekabsons MB, Nagle DG, et al. *J. Nat. Prod*. , 2010; **73:** 1868.
21. Oduola T, Adeniyi FA, Ogunyemi EO, Bello I S, et al. *J. Med. Plants Res*. , 2007; **1:** 1-4.
22. Sartoretto A, Machado ALM, Delarmelina C, Figueria GM, et al. *Braz. J. Microb*., 2004; **35:** 275-280.
23. Abdel-Latif A, Koenig G.M, Fisch K.M, Höller U, et al. *J. Nat. Prod.,* 2002; **65:** 1605-1611.