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Use of *Maerua Decumbens* as a Natural Coagulant for Water Purification in the Dry Lands of Kenya

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ABSTRACT: Indigenous management and utilization of naturally occurring tree species and shrubs/lianas is not a new culture worldwide. Various communities in the world use their indigenous tree /shrub / liana species to meet their needs for food (human and livestock), shelter and medicine among other diverse wood and non-wood forest products. Introduction of new exotic species has eroded the importance of some of these important indigenous plant species to great extends. It is however, important to consider that while these exotic species have multiple uses, most of them are not well adapted to our arid and semi-arid regions hence the need to promote the management and sustainable use of the indigenous species. *Maerua decumbens* is a shrub or woody herb species in the Capparaceae family and grows to a height of 0.5 to 3m with a large swollen root. It mostly occurs naturally in the arid and semi-arid areas in Kenya and is used traditionally by rural communities for medicinal and water purification purposes. Members of the *Mearua species* are indicated as poisonous and probably a health risk and yet some of the communities chew the roots of *Mearua decumbens* against thirst and also use them for purifying water (Beenje H.1994). The study was done to enhance the use of *Maerua decumbens* as a natural coagulant for water purification by determining whether the plant used for water purification in Mutha in Kitui County is toxic or not. A reconnaissance survey was done to confirm its use for water purification and toxicity tests were done to determine the safety of the plant for human utilization as a natural coagulant for water purification. The results of the study revealed that *M.decumbens* is completely safe for human consumption and does not have any heavy metals that pose a risk to human health.

Keyword: Indigenous, natural, coagulant, utilization, safety

INTRODUCTION

Background

Local indigenous trees, shrubs and herbs in the arid and semi-arid lands (ASALS) play a key role in the livelihood of the ASAL's populations. This is by supplementing the food and fodder resources during dry seasons, providing medicine and enhancing environmental conservation (Ngina *et a.l*, 2004). Despite the multiplicity of these species and the multiple roles in their uses, their full potential has not been exploited. This is mainly because their uses are based on local knowledge with little or no scientific validation.

In the past, government policies were geared towards mobilization of human and financial resources in the country for adoption of new technologies in all section of the economy. This was to accelerate the country's development process and in the process indigenous technologies were ignored. After decades of modernization policies, it has been realized that a large proportion of the population have been left out due to many factors among them socio-economic, cultural and literacy levels. Most of these groups still rely on old traditional indigenous technologies to

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meet their daily need hence the recent policy shift towards validating the viability of some of the indigenous technologies applied in various sectors with the view of promoting them. Since some of these technologies are mainly applied by the poor section of the population, their integration into research and extension will generate greater multiplier effects in the improvement of the livelihoods of the poor local communities in developing countries.

Water is a basic need and an important catalyst necessary to accelerate both economic and social development in the country (Macharia, 2014). It is therefore necessary to provide safe and clean water for use by various stakeholders. In Kenyan rural areas where water infrastructure is lacking, provision of clean water is quite a challenge. This leaves most residents to rely on overstretched traditional sources such as dams, rivers and seasonal streams which often provide unsafe domestic water. Technologies which make such water safer for human use are therefore required. Introduction of commercial water purification technologies has eroded the importance of plant coagulants. However, many residents of the rural ASALs still rely on natural coagulants such as found in *Moringa oleifera* and indigenous species such as *Mearua decumbens*, to purify water for domestic use. Beentje (1994) indicates that the shrub is well distributed in Kenya. Seed kernels of Moringa have been used traditionally for household water treatment in Sudan and Indonesia. *Moringa oleifera* and *Moringa stenopetala* have been used in water treatment in Malawi (Sutherland et al, 1990).

Maerua decumbens is one of the indigenous shrubs or woody herb species in the Capparaceae family and grows to a height of 0.5 to 3m with a large swollen root. It occurs naturally in the arid and semi-arid areas in Kenya. It does well in altitudes ranging from sea level to 1800m above sea level especially in deciduous or semi-green bush land, bushed grassland or wooded grass land. In dry areas, it is often found near seasonal rivers, lakes or on alluvium. It is conspicuous on burned grassland where it regenerates quickly from its thick woody root.

Boiled fruits are used as food during times of severe drought and famine. Members of the Pokot use a leaf decoction on sore points (Beentje, 1994) The Kamba use the leaf decoction to treat oedema or the swelling of the body.

Justification

Most of the arid and semi-arid areas of Kenya experience long drought. During these periods, the availability of water for domestic and other uses is a problem to most communities living in such areas. The rivers in these areas are mainly seasonal and only few are permanent or semi-permanent. Therefore, during the dry season any water available from whichever source is of great use to the people. After rains in the dry lands, there is a lot of runoff which contribute to turbid water gathering in the rivers, streams, ponds and dams. Such water is very valuable to the dry land communities, and for good health; it needs to be treated before domestic use.

In most of the rural areas, water purification services or chemicals are not available and those available are very expensive hence alternative indigenous methods for water purification are necessary. There is need to focus on natural coagulants from plants because they are cost effective, easily available and possess less adverse effects on human health. Provision of clean water in the dry lands is a challenge and pollution of surface and underground water resources is a serious problem. Traditional water purification methods may vary from community to community. Some community members in Mutha (Kitui County), Marigat (Baringo County), in Turkana County and Tana River County indicated that they use the roots of *Maerua species* to purify muddy water available during the rains. However, some of the Mutha community members indicated a traditional believe that the species is poisonous. Maerua species have been reported to be poisonous and probably a health risk (Noad T and Bernie A, 1989), and yet some of the communities chew the roots of *Maerua decumbens* against thirst and use them for water purification (Beenje H., 1994). More so, the use of this particular species by most local people as a source of fruit, medicine, fodder and coagulant for dirty water has been noted. Therefore the safety of such plants needs to be verified and validated. It was therefore, considered necessary to enhance the use of *Maerua decumbens* as a natural coagulant for water purification by determining whether the plant as used for water purification in Mutha in Kitui County is toxic or not.

Broad objective

To enhance the use of *Maerua decumbens* as a natural water coagulant in the dry lands of Kenya

Specific Objective

- To investigate the cytotoxicity of *maerua decumbens* roots used for coagulation of water for human consumption.

Activities

- Evaluation of the in vitro safety of aqueous and partitioned extracts of *Maerua decumbens.*
- Evaluation of the in vivo safety of aqueous extracts of *Maerua decumbens.*

- Determination of presence of heavy metals in Maerua root extract used for water purification.

STUDY METHODOLOGY

Household Interview

The traditional use of *Maerua decumbens* was noted among the Kamba community in Kitui during social forestry extension activities implemented by Social Forestry Training Project funded by Japan International Co-operation Agency (JICA) running through the period 2002 to 2007. A checklist was used and over 100 different household respondents from the selected sites namely Kitui and Baringo were engaged in discussion to confirm whether the species was known as a natural water coagulant, whether it was being used by other communities apart from the Kamba in Kitui County, the process of use and its status of use among the local dry land communities. Samples of M. decumbens were also collected and preliminary tests undertaken in the laboratory to confirm its mode of use.

In vitro safety evaluation

Plant sample collection and preparation

Collection of root samples for the study was done in Kitui only due to limitation of costs of toxicity tests. The roots were uprooted carefully and taken to KEMRI laboratory for traditional medicine where the plant root samples were cut into small pieces, dried at room temperature and ground into a fine powder. Extraction was done using double distilled water and the filtrate was freeze-dried. The biochemical responses of cells after exposure to plant water extracts; total aqueous and solvents partitioned with petroleum ether were observed and reported using MTT dye (3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide). The study design was Cell based assay.

Procedures for preparation of 1000 µg/ml Maerua concentration

The following procedures were used for preparation of Maerua concentration

- Weighed 0.01g (10mg) of extract in 15ml centrifuge tube
- Added 1ml of phosphate buffered saline (PBS) solution and dissolved the extract using a vibrating mixer
- Maintenance media (MM) was added up to 10ml mark
- The mixture was filtered using 0.22µm membrane syringe filter and filtrate labelled as 1mg/ml (1000µg/ml)
- The labelled filtrate was kept at $^{-}20^{o}$C freezer until required for use

Materials used for Cytotoxicity assay

1. 96 well plates suitable for tissue culture
2. Multichannel pipette's
3. 96 well spectrophotometer
4. Cell line (Vero cells (ATCC®)
5. ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003
6. Foetal Bovine serum
7. Dimethyl Sulphoxide (DMSO)
8. MTT dye
   i. $NaHCO_3$
   ii. HEPES

Cell Line Culture Procedure

This test was carried out alongside the promega protocols and application guide. Vero cell line passage 30 was used. The cells were cultured on ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003 supplemented with 10% fetal bovine serum, L-glutamine (2mM) and 1% penicillin-streptomycin in static 75 cm^2 T-Flask (GIBCO, USA). The cells were incubated in a humidified enclosure with 5 % CO_2 at 37^0C.

Cell Cytotoxicity Assay

Cells were plated in a 96- cell plate 2×10^4 and in a suspension of 100µl. The cells were left overnight (24 hours) to adhere before being exposed to the drug formulation at a starting concentration of 1000µg/ml and subsequent three fold serial dilutions. 10µl of 5mg/ml MTT reagent in sterile PBS was added directly to the cells. The cells were further incubated for 4 hours for formation of insoluble purple formazan from yellowish MTT by enzymatic reduction of tetrazolium salt to formazan. The insoluble formazan was solubilized by adding 100µl of DMSO after removal of supernatant. The optical density of solution was measured at 562 using microplate reader (Multiskan EX, Labsystems).

Methodology for In vivo safety evaluation

Materials

The main materials used include aqueous extract of *Maerua* spp and distilled water. The animals used for the evaluation of in vivo safety were 12 Male Swiss Albino mice, 7 weeks old

Procedure

The OECD Acute Toxic Class[1] method for categorizing the acute oral toxicity of substances into pre-determined lethal dose cut-offs was used with modifications.

[1] OECD, 'OECD GUIDELINE FOR TESTING OF CHEMICALS'.

The extract was diluted using distilled water into the required concentrations. The mice were divided into four groups of three members each. Four dose levels were chosen, 50, 300, 2000 and 5000 milligrams/kilogram of body weight (mg/kg) and allocated to the four animal groups. The respective doses were administered singly via the oral route after fasting the animals for four hours. The animals were closely and periodically observed, after administration of the doses, and thereafter daily for seven days for signs of toxicity. On the seventh day after the administration, the animals were killed humanely and a thorough *post mortem* examination carried out on the carcasses.

Methodology for determination of heavy metals in the water samples

Samples for Heavy metal analysis were prepared in Karura laboratory through grinding some portion of the root, weighing and mixing with one liter of distilled water. Elemental analysis was done. Heavy metals for which Maerua was tested in water samples analysed include: Copper (Cu), Lead (Pb), Zinc (Zn) , and Arsenal (As).

Apparatus/equipment

1. Digestion block
2. Volumetric flask
3. Conical flasks
4. Micro pipettes
5. Digestion tubes
6. Analytical balance
7. Hot plate/Magnetic stirrer
8. grinder
9. Atomic Absorption Spectrophotometer
10. Reagents
11. Sulphuric acid – H_2SO_4 (93-98%)
12. Nitric acid (HNO_3)
13. Hydrated Copper sulphate – $CuSO_4H_2O$ (AR grade)
14. Potassium Sulphate (K_2SO_4) –or anhydrous Sodium Sulphate (Na_2SO_4) (AR grade)
15. Commercial manufactured standards of, Cu, Pb, Zn and As stock solution of 1000ppm

Procedure

1. 10ml of water sample weighed and put in each digestion tube.
2. 0.5 g Copper Sulphate and 5g Potassium Sulphate were added.
3. 20 ml conc. H_2SO_4 was added in each tube.
4. The solution was heated gently at 110°C for 1 hour until frothing ceased.
5. The temperature was raised to 360°C and continued heating for 2 hours to attain complete oxidation.
6. Contents were allowed to cool for about 30 minutes.
7. 25 ml of distilled water was added and the contents transferred to 50 ml volumetric flask.
8. Pb, Cu, Zn and As in the digests were determined as outlined here below.

Copper Analysis

1. Standard stock 100 ppm was made from commercially manufactured Cu 1000 ppm stock.
2. Working Copper standard series as follows: 0, 1.0, 2.0, 4.0, 6.0, 8.0 and10.0 ppm solution were prepared by diluting stock solution of 100 ppm Cu.

Procedure

Copper standard series, blank digests and samples were aspirated into the atomic absorption spectrophotometer and the absorbencies were measured. A calibration curve was plotted from the readings of the standard series and the concentration of the unknown of was determined.

Calculations

The mean blank value was subtracted from the unknowns; this gave a value for corrected concentration (= C in subsequent calculations).

$$\text{Cu in sample (ppm)} = \frac{C * ppm\ solution * df}{w}$$

Where C = the corrected concentration of Cu in the sample; ppm solution = graph reading; df = dilution factor; w = weight of dry the sample.

Lead Analysis

Standards

Standard stock 1000 ppb was prepared from commercially manufactured Pb 1000 ppm stock.

Working lead standard series were also prepared as follows: 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppb solution by diluting stock solution of 1000 ppb Pb and adding a drop of concentrated HNO_3.

Procedure

Lead standard series, blank digests and samples were aspirated into the atomic absorption spectrophotometer and the absorbencies were measure.

Calibration curve was plotted from the readings of the standard series and the concentration of the unknown was determined.

Calculations

By Subtracting the mean blank value from the unknowns; this gave a value for corrected concentration (= C in subsequent calculations).

$$\text{Pb in sample (ppb)} = \frac{C * ppm\ solution * df}{w}$$

Where C = the corrected concentration of Pb in the sample; ppb solution = graph reading; df = dilution factor; w = weight of dry the sample.

Zinc Analysis
Standards

Standard stock of 1000 ppb was made from commercially manufactured Zn1000 ppm stock. Working Zinc standard series were prepared as follows: 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppb solution by diluting stock solution of 1000 ppb Zn and adding a drop of concentrated HNO_3.

Procedure
Zinc standard series, blank digests and samples were aspirated into the atomic absorption spectrophotometer and the absorbencies measured.

Calibration curve was plotted from the readings of the standard series and the concentration of the unknown was determined.

Calculations

By Subtracting the mean blank value from the unknowns; this gave a value for corrected concentration (= C in subsequent calculations).

$$\text{Zn in sample (ppb)} = \frac{C * ppm\ solution * df}{w}$$

Where C = the corrected concentration of Zn in the sample; ppb solution = graph reading; df = dilution factor; w = weight of dry the sample.

Arsenic Analysis
Standards

Standard stock 1000 ppb was made from commercially manufactured As 1000 ppm stock.

Arsenic working standard series were prepared as follows: 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ppb solution by diluting stock solution of 1000 ppb As and adding a drop of concentrated HNO_3.

Procedure
Arsenic standard series, blank digests and samples were aspirated into the atomic absorption spectrophotometer and their absorbencies were measured.

A calibration curve was plotted from the readings of the standard series and the concentration of the unknown was determined.

Calculations

By Subtracting the mean blank value from the unknowns; this gave a value for corrected concentration (= C in subsequent calculations).

$$\text{As in sample (ppb)} = \frac{C * ppm\ solution * df}{w}$$

Where C = the corrected concentration of As in the sample; ppb solution = graph reading; df = dilution factor; w = weight of dry the sample.

RESULTS AND DISCUSSION

Traditional use of Maerua *decumbens* for water purification

From the focused group discussions and field observations, it was clearly noted that the species was being used for coagulation of dirty water obtained from dams, rivers and pools of surface runoff water. Community members in Kitui referred to it as Munatha while in Baringo, the Kalenjins referred to it as Chepluswa and the Turkana named it Eerut. Over 90% of respondents in each site confirmed to have used the plant to clean dirty water. For the mode of use among the different communities, it was clearly indicated that the bark of the tuberous root of *M. decumbens* was peeled and used for cleaning water by stirring dirty water to coagulate the suspended dirt. Community members in Baringo were found using Maerua root for cleaning water collected from dams for laundry use.

In comparison to Alum, the community members in Baringo indicated that Maerua was more preferred to Alum because it was easy to access and no financial costs were incurred unlike for Alum. More so, they also indicated that water treated with Maerua root was suitable for cooking milk tea while Alum treated water could not be used for milk tea since it caused the milk to form curds. Such observations were supported by some studies which indicated that most water treatment chemicals are costly to access since most of them are imported. It was also argued that Aluminum has been indicated to be a causative agent in neurological diseases such as pre-senile dementia

and that there was a fear that ingestion of aluminum ions may induce Alzheimer's disease (Binayke *et al*, 2013)

During the surveys and focused group discussions, samples of Maerua root were collected and using dirty surface runoff water samples, simple tests on the capacity of *M. decumbens'* root to coagulate dirty in water were done in the Laboratory in KEFRI Karura Regional Centre for Forest Products. The root was ground in to powder (Figure 1) dissolved in water and the mixture was dried using evaporation method to get more refined powder (Figure2) which was used to clean the dirty surface run off water (Figure 3).

Figure1: Root of *M.decumbens* ground in to powder

Figure 2: Refined powder of *M. decumbens* obtained through evaporation

M. decumbens was confirmed to have the capacity to coagulate suspended dirty in water hence making it clean enough to wash clothes and for other domestic uses (Figure 3).

Figure3: Dirty surface run off water cleaned using refined *Maerua* powder

However, the community members interviewed indicated that the status of use of *M. decumbens* root for water purification was going down with emergence of artificial water coagulants such as Alum and other artificial water purifiers used in form of tablets and liquids.

In vitro safety evaluation of *M. decumbens*

MTT assay is a rapid and high accuracy colorimetric approach and is widely used to evaluate cell growth and cell cytotoxicity, with more emphasis in the development of new drugs. Membrane integrity is measured by determining mitochondrial activity through enzymatic cleavage on the reduction of tetrazolium salt to formazan.

The cell growth profile after treatment with the extract in MTT Assay is presented in Table 1 and Figures 1&2 below. The extracts gave IC50 values above 1000 µg/ml. The detachment capability of the extracts was not observable even on the cells that received the highest concentration (1000 µg/ml indicating that it is safe to use *M. decumbens* for coagulation of dirt in water for domestic use.

Table1: Optical Density (Absorbance)

	1	2	3	4	5	6
A	0.54	0.427	0.06	0.419	0.43	0.06
B	0.501	0.448	0.062	0.435	0.444	0.065
C	0.481	0.462	0.058	0.407	0.426	0.064
D	0.47	0.432	0.042	0.409	0.434	0.073
E	0.471	0.436	0.073	0.408	0.435	0.074
F	0.455	0.435	0.066	0.411	0.417	0.065
G	0.48	0.48	0.056	0.49	0.484	0.097
H	0.529	0.45	0.065	0.537	0.537	0.08

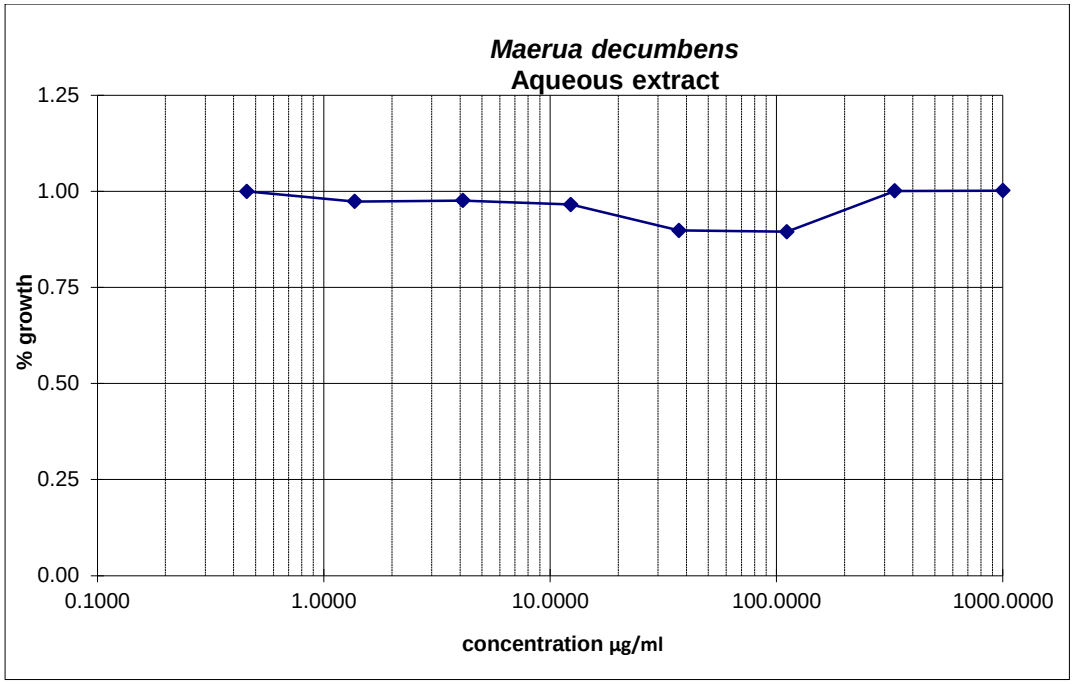

Figure1: The effect of *Maerua decumbens* aqueous extract assay on Vero cell grow

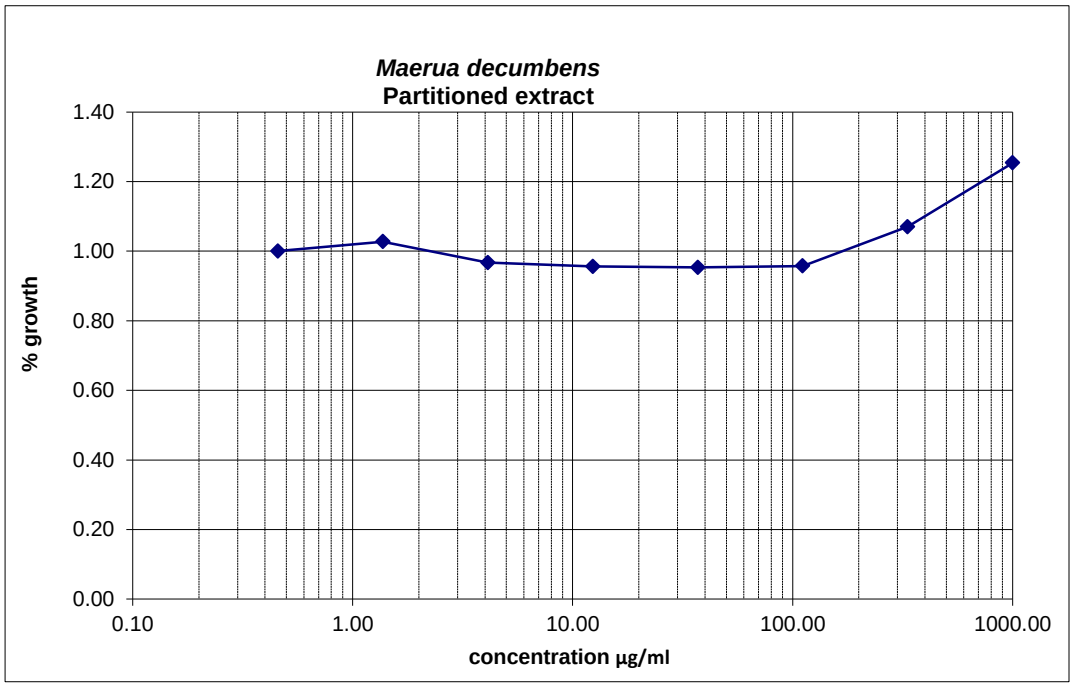

Figure 2: The effect of *Maerua decumbens* partitioned water extract of Vero cell growth

From the results obtained, it was found that both aqueous and partitioned extracts of *Maerua decumbens* had no significant reduction in the number of viable cells at concentration of up to 1000µg/ml. Statistically the IC50 value was not observable within the test concentration limits that were assayed.

In most instances, concentrations of drugs or other substances above 100µg/ml which do not show/display any cytotoxic activity are considered quite safe. In this respect *Maerua decumbens* water extract can be reported as safe to animals and human subjects as per observations made during this study. Its reported use in traditional turbid water purification systems in arid and semi-arid areas is therefore

supported by the findings of this study. Indeed indigenous systems should be improved and adopted to better the lives of inhabitants/residents of the dry land local communities. Such indigenous plans can also be commercialized to economically benefit the communities who own this knowledge. Boiling of such water is however encouraged at this stage to ensure its safety before consumption.

In vivo safety evaluation for *M. decumbens* extracts

There were no clinical signs of toxicity in any animal or any dose level over the observation period. There were equally no gross lesions observed on any of the carcasses on *post mortem* examination.

Analysis of *M. decumbens* extracts for Heavy metals

From the results of analysis, commonly encountered heavy metals were not detected from the water samples even at very high concentration. Therefore, Maerua lacks heavy metals including Lead, Copper, Zinc and Arsenic hence it does pose any hazard to animal and human health.

CONCLUSIONS AND RECOMMENDATIONS

M. decumbens is used traditionally for coagulating dirt in surface run-off, river and dam water. The plant extract is neither cytotoxic to Vero cells at tested dose levels nor toxic to laboratory mice after a single dose at the tested levels. The plant species is safe for human consumption as observed with the in vivo safety evaluation. *M. decumbens* does not have heavy metals hence safe for both animal and human.

The extract/plant species can therefore be considered safe for use in indigenous water purification systems. Further studies on phytochemical analysis are suggested to narrow down on the chemical compounds responsible for the water sedimentation activity. Additionally, antimicrobial studies can be done to determine whether *M. decumbens* have double activity on sterilization and purification of turbid water.

The community members interviewed also suggested that the plant should be processed in to powder form for easy use because they would still prefer to use the plant they have known for years as a water purifier rather than using the artificial coagulants and water purifiers.

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GLOSSARY

In vivo- studies in which the effects of various biological entities are tested on whole living organisms usually animals (including humans) and plants.

In vitro- studies performed with cells or biological molecules outside their normal biological context e.g. in test-tubes, flasks, Petri dishes *etc.*

Vero cell - lineages of cells used in cell cultures.