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Toxicity assessment and modelling of *Moringa oleifera* seeds in water purification by whole cell bioreporter

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ABSTRACT

Moringa oleifera has been used as a coagulation reagent for drinking water purification, especially in developing countries such as Malawi. This research revealed the cytoxicity and genotoxicity of *M. oleifera* by *Acinetobacter* bioreporter. The results indicated that significant cytoxicity effects were observed when the powdered *M. oleifera* seeds concentration is from 1 to 50 mg/L. Through direct contact, ethanolic-water extraction and hexane extraction, the toxic effects of hydrophobic and hydrophilic components in *M. oleifera* seeds were distinguished. It suggested that the hydrophobic lipids contributed to the dominant cytoxicity, consequently resulting in the dominant genotoxicity in the water-soluble fraction due to limited dissolution when the *M. oleifera* seeds granule concentration was from 10 to 1000 mg/L. Based on cytoxicity and genotoxicity model, the LC_{50} and LC_{90} of *M. oleifera* seeds were 8.5 mg/L and 300 mg/L respectively and their genotoxicity was equivalent to 8.3 mg mitomycin C per 1.0 g dry *M. oleifera* seed. The toxicity of *M. oleifera* has also remarkable synergistic effects, suggesting whole cell bioreporter as an appropriate and complementary tool to chemical analysis for environmental toxicity assessment.

1. Introduction

As one of the key water purification processes, coagulation binds the colloidal particles and bacteria, allowing an electrostatic precipitation of contaminates from solution, the cost-effective consideration of which is distinct in developing countries, such as Malawi. The seeds of *Moringa oleifera*, which is a tropical tree commonly found in parts of Africa, India, Malaysia, Sir Lanka and America, has been widely used as a coagulant in terms of the powder extract (Diaz et al., 1999; Ghebremichael et al., 2005; Madsen et al., 1987; Muyibi and Evison, 1995a; Ndabigengesere et al., 1995). Evidence suggests that the bacterial colloids have been reduced by a percentage of 90–99% by the action of *M. oleifera* seeds (Sutherland et al., 1990). Further research has shown that *M. oleifera* has active diametric cationic proteins and a molecular weight of 12–14 kDa, a large cation (Ndabigengesere et al., 1995). The seed extract works by adsorption of colloids and subsequent charge neutralisation of the resulting compound, allowing for effective precipitation out of solution. The study showed that compared to alum, the optimum dosage of

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shelled *M. oleifera* was similar at 50 mg/L. In the case of seeds with the shells remaining the effective dosage increases tenfold (Ndabigengesere et al., 1995). The normal dose used to treat water of turbidity less than 100 NTU is in the range of 100–200 mg/L (Muyibi and Evison, 1995b; Nkurunziza et al., 2009; Sutherland et al., 1990). For highly coloured water (such as commonly found in Malawi) doses of up to 250 mg/L may be required, which could place the supernatant in the toxicity range. From Sutherland's work at Thyolo Water Treatment Works in Malawi, the dosage of *M. oleifera* seeds as a coagulant ranged from 75 to 250 mg/L, reducing the high turbidity of river water from 270 to 380 NTU down to 4 NTU (Sutherland et al., 1994).

The bioactive agent was shown to be a steroidal glycoside-strophantidin and the seed powder reduced total microbial and coliform counts by 55% and 65% respectively (Eilert et al., 1981). The 4-(α-L-rhamnosyloxy-benzyl) isothiocyanate was isolatyed as the active antimicrobial component in *Moringa* seeds (Eilert et al., 1981). The chemical constituents of the seeds contains 4-(α-L-rhamnosyloxy) benzyl isothiocyanate, 4-(L-rhamnosyloxy) phenylacetonitrile, 4-hydroxyphenylacetonitrile, 4-hydroxyphenyl-acetamide, 4-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate Roridin E, Veridiflorol, 9-Octadecenoic acid, O-ethyl-4-(α-L-rhamnosyloxy) benzyl carbamate, niazimicin, niazirin, β-sitosterol, glycerol-l-(9-octadecenoate), 3-O-(6-O-oleoyl-β-D-glucopyranosyl)-β-sitosterol and β-sitosterol-3-O-β-D-glucopyranoside (Fahey, 2005). A further study (Santos et al., 2009) isolated the coagulant of *M. oleifera* by separating the lectin from the seeds to evaluate the hemagglutinating activity in comparison to alum. This was shown to be unique while the coagulant activity was comparable to alum. Other research also demonstrated that the active component of *M. oleifera* was neither protein nor a polysaccharide (Okuda et al., 2001).

Nevertheless, complex components of *M. oleifera* have also raised concerns on its potential toxicity (Chivapat et al., 2012; Kavitha et al., 2012). The toxicity of aqueous *M. oleifera* extraction was estimated since 1990's (Asare et al., 2012; Awodele et al., 2012) and both acute and chronic impacts have been reported (Chivapat et al., 2011). Mustard oil glycosinolates are abundant in *Moringaceae*, which break drown to a glucose sugar, sulphate, isothiocyanates (mustard oils) or an organic nitrile. Isothiocyanates are a skin irritant and irritate the mucous lining of the gastrointestinal tract. Kidney and liver damage may also result from this fraction along with inhibiting the uptake of iodine for the thyroid (Fuller and McClintock, 1986). *M. oleifera* is also reported to contain three mustard seed oil glycosides. Glucosinolates are precursors or organic isothiocyanates that break down to produce giotrogenic agents that cause hyperplasis and hypertrophy of the thyroid gland. In addition, isothiocyanates have shown to cause gastrointestinal tract lesions in cattle (Majak, 2001). Besides, further research has revealed that higher level toxicity of *M. oleifera* leaves was observed in the ethanolic extraction, with significant negative effects on rat cells by inhibiting lipid peroxidation (Ouedraogo et al., 2013).

Significant toxicity effects have been investigated previously with respective toxicity assay. Oluduro and Aderiye dosed *Moringa* seed treated water (1–10 mg/mL) to male albino rats daily for 21 days, and the results suggested that prolonged consumption of water treated with greater than 2 mg/L of *M. oleifera* seed constitute liver infarction (Oluduro and Aderiye, 2009). The oxygen uptake of *T. pyriformis* was affected by 5 mg/L *M. oleifera* seeds solution, whereas the 96-h LC_{50} for fish guppies (*Poecilia reticulata*) was 196 mg/L (Grabow et al., 1985). Fed to 33 days old Hooded-Lister (Rowett strain) rats at a dosage of 50 g and 100 g of seed protein per kg of meal, high toxicity could be observed at an equivalent intake of 1.9 g of *M. oleifera* per day (Grant et al., 1991). The assessment of coagulant *M. oleifera* lectin (cMoL) on moth flour (*Anagasta kuehniella*) suggested that cMoL at 1% w/w could increase the mortality by 27.6%, indicating that the activity of cMoL is a carbohydrate-lectin action on the digestive tract (Ramalho de Oliveira et al., 2011). The effects of *M. oleifera* on the sexual behaviour of mice was also found in terms of increasing lumen formation and epididymal maturity (Cajuday and Pocsidio, 2010). All the research required toxicity assessment with mammalian cells or living animals, restricted by the laborious cultivation, high cost and long test period, raising the demand of fast assessment methods for the toxicity of *M. oleifera*.

In this research, a rapid, cheap and easy approach was explored to estimate the toxicity of *M. oleifera*, applying whole cell bioreporter ADPWH_recA (Song et al., 2009). As a practical tool for drinking water monitoring and quality assessment in Africa, whole cell bioreporter can effectively assess the cytoxicity and genotoxicity. The evaluation on different extractions, including water, ethanol and hexane, has revealed the impacts of respective components of *M. oleifera* on cytoxic and genotoxic behaviour of living bacteria. The mechanisms of *M. oleifera* toxicity were also characterised and analysed by a cross-regulated SOS response model.

2. Material and methods

2.1. Moringa oleifera *chemical analysis and extraction*

2.1.1. *Chemical analysis*

The collected seeds of *M. oleifera* were ground under liquid nitrogen in a pestle and mortar and then freeze dried for 24 h. Before chemical analysis and further treatment, the seeds samples were air dried at room temperature and pulverized into granules. All the units of the components concentration were based on the dry weight of the seeds if not specifically mentioned.

For the protein analysis, 1.0 g of *M. oleifera* granules was re-suspended in 0.8 mL phenol solution (equilibrated with 10 mM Tris HCl, pH 8.0) and 0.8 mL sodium dodecyl sulphite (SDS) buffer (30% sucrose, 2% SDS, 0.1 M Tris–HCl, pH 8.0, 5% 2-mercaptoethanol). After mixing, the phenol phase was separated by centrifugation at 10,000 rpm for 3 min, and transferred into fresh tubes with the addition of five volumes of cold methanol and 0.1 M ammonium acetate. Precipitated proteins were recovered and then washed with cold methanolic ammonium acetate twice and cold 80% acetone twice. The final pellet was dried and dissolved in phosphate buffered saline. The sample was then analysed using Bradford's assay against BSA as a standard, to determine total protein concentration.