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Full Length Research Paper

Application of phytodisinfectants in water purification in rural Cameroon

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Findings from a preliminary lab-scale study show strong potentials of phytodisinfectants as a low-cost, appropriate and ecological alternative technology in purifying water in rural Cameroon. A survey of plants used in water purification in Bamenda, Cameroon, indicated that there are many plants used in water treatment. A rapid screening on the coagulative and disinfection potential of four most frequently used plants was carried out on; *Moringa oleifera*, *Jatropha curcas*, calyx of *Hibiscus sabdarifa*, sclerotium of *Pleurotus tuberregium* against their crude methanol extracts, aluminum sulphate and sodium hypochlorite controls on turbid surface water samples. A beaker experiment with varying weights (0.5 to 5 g) of dried pulverized plant materials and alum (control) were placed in 200 ml each of the three-turbid water samples and left for thirty minutes retention time. A 95% reduction in bacterial loads of the water samples by *M. oleifera* in fifteen minutes residence time was observed. *J. curcas* seeds, as well as *H. sabdarifa* calyx also reduced the bacterial loads between 75 to 90%. All the plant extracts except *P. tuberregium* inhibited an *Escherichia coli* isolate from the turbid water with highest zone of inhibition (15 mm) recorded for *M. oleifera* seed extract. The inhibition zones produced by three of the plant extracts were comparable to aluminum sulphate (6 mm) and sodium hypochlorite (17 mm). Crude methanol extracts from *M. oleifera* seeds, *J. curcas* seeds and *H. sabdariffa* calyx used directly on turbid water drastically reduced the total aerobic mesophilic bacterial counts far more than the unextracted plant powders. The turbidity of both phytodisinfectant and alum treated water samples drastically reduced while no turbidity reduction was observed with sodium hypochlorite treated water samples. The pH of alum treated water was observed to decrease from neutral to 5.0 while pH of phytocoagulant treated water was 7.0. This report suggest that *M. oleifera* seeds, *J. curcas* seeds and calyx of Hibiscus sabdariffa posses both phytodisinfectant and phytocoagulant property in water purification. Scerotium of *P. tuberregium* poses only phytocoagulant (mycocoagulant) activity. Plant materials can be used as phytocoagulants and phytodisinfectants in treating turbid water and can be applied in wastewater treatment. Further studies on the application of Phytodisinfectants in domestic water purification, especially the phytodisinfection potentials of *M. oleifera* are exigent.

Key words: Phytodisinfectants, phytobiotechnology, phytocoagulants, bacteria turbid water, plants, *Moringa oleifera*, Cameroon.

INTRODUCTION

Safe drinking water and adequate sanitation are essential for human health and dignity. However, 1.2 billion people do not have (Pritchard et al., 2009; UNICEF, 2009). Approximately, 2.5 billion people in the world lack adequate sanitation facilities (UNICEF, 1993; UNEP, 2002; Zhang et al., 2006; UNESCO, 2007; UNICEF, 2009). Waterborne and water related diseases such as diarrhea, typhoid, cholera and drancunculiasis are fast becoming endemic in certain parts of Africa (Cheesbrough, 1984; Yongabi, 2004; Pritchard et al., 2009). Yet, the present well documented technologies used in water treatment such as reverse osmosis, ion exchange, uv sterilization, aluminum sulphate and chlorine are becoming unsustainable, unecological,

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Figure 1a. *Moringa oleifera* seeds identified in Cameroon and can be used for community water purification.

Figure 2. *Jatropha curcas* (Physic nut) (Yongabi, 2004) and Sclerotium of *Pleurotus tuberregium* (Yongabi, 2004).

Figure 1b. *Moringa oleifera* grows rapidly from seed (Yongabi, 2004).

expensive to run, managed and maintained, particularly in Africa (Pritchard et al., 2009). For example, Chlorine is known to produce trichloromethane, a cancer precursor (Yongabi, 2004) while Aluminum sulphate has been linked to Alzheimer's disease (Zhang et al., 2009). Furthermore, the cost of purchasing synthetic coagulants and disinfectants is in hard currency leading to high pricing for treated water in Africa (Kebreab et al., 2005) (Figure 1a and b). Simple technologies such as the application of plant coagulants such as Moringa oliefera to treat water has been extensively reported (Oliver, 1959; Jahn, 1981; Muyibi et al., 2002; Yongabi, 2004; Pritchard et al., 2009).

Interest in isolating and purifying bioactive *Moringa oleifera* coagulant ingredient has grown and outweighed efforts on taking inventory of other potential plant coagulants and disinfectants. The most important step in water treatment is disinfection. Attention has been focused on screening plants for coagulant activity (Eilert, 1978; Jahn, 1981; Muyibi et al., 2002a; Kebreab et al., 2005; Amir et al., 2010), but not all coagulants are disinfectant. The flora of Africa is rich with a lot of medicinal plants and Macro fungi which people in the rural areas are quite familiar (Figure 2). Sofowora (1982) and Yongabi (2004) reported that Africa has as much as 300, 00 medicinal plants while Chang (1993) reported that the world mushroom biodiversity is as much as 1.5 million species. There is, therefore, an urgent need to explore and utilize these rich biodiversity through researches that could translate to direct benefit to humankind (Yongabi, 2009). Plant disinfectants could provide useful insight for the production of natural disinfectants and coagulants which are environment friendly and with much reduced risk of handling.

The ultimate purpose of this research was to carry out an inventory of plants used as disinfectants in rural Cameroon, conduct an *in vitro* evaluation of crude plant powders and solvent extracts on directly disinfecting turbid surface water from Bambui, Cameroon.

MATERIALS AND METHODS

Water sample collection

Water samples collected included turbid surface water sources in Bambui, Cameroon. Dirty water flowing in Gutters and water from a stream around the Bambui settlement, kitchen sink samples, as well as septic tank outflows was collected. The appearance/cloudiness of these water samples were noted by visual observation (Burns and Van, 1974).

pH analysis

The PH was recorded using a test strip (combi 9 test strips) used at the phytobiotechnology research laboratories for routine urinalysis.

Microbial analysis of untreated and treated water samples

Escherichia coli, coliforms and Total aerobic mesophilic bacterial counts were enumerated on Eosin Methylene blue, MacConkey and Nutrient agars respectively (Harrigan and Mccance, 1976). A milliliter of the turbid water samples were aseptically diluted serially up to three fold. This was done according to methods of APHA (1983), Cheesbrough (1984) and Yongabi (2004). All these were done before and after coagulation and disinfection.

Plant collection and processing

Seeds of *M. oleifera*, *Jatropha curcas*, calyx of *Hibiscus sabdarifa* and Sclerotium of *P. tuberregium* were previously obtained from Fulani settlements around Sabga in the North West Region of Cameroon, dried, then pulverized separately using a clean pestle and mortar and stored in brown paper bags ready for the test. The identities of the plants were previously confirmed by Botanist at The University of Yaounde, Cameroon.

Beaker tests to determine disinfection and coagulation using crude plant powders

Graded weights (0.59 to 59) of the pulverized plant materials and Alum were each added to 200 mls of the wastewater samples in beakers. Increased weight s (g) from 0.5 to 5.0 g of each of the plant material was mixed in a small quantity of the turbid water to form a paste and then mixed finally with the water samples in the beakers. The same procedure was done for alum, sodium hypochlorite and a turbid water sample in a beaker (200 ml was allowed to stand in a beaker for 24 h as control. The coagulative effect and change in bacterial counts were recorded (Kebreab, 2005; Pritchard et al., 2009).

Bulk methanol extraction procedure for plant powders

A cold methanol extraction was then carried out on 100 g each of the powders by steeping each of the powders in 250 ml of methanol for 24 h. Gravity filtration was carried out using what man filter paper No. 13 and solvent evaporated at room temperature in a fume cupboard.

Preliminary antibacterial determination of crude methanol extracts on an *E. coli* isolate from surface water in Bambui, Cameroon

This was carried to confirm if the bacterial reduction previously observed is due to inherent disinfectant ability of the plants. One hundred (100) mg of each of the extract was suspended in 1 ml of distilled water. The extracts were now tested for their *in vitro* antibacterial activity using the Agar diffusion Method using *E. coli*, previously isolated from the turbid water rather than using synthetic turbid water as previously carried out by most researchers. The choice of *E. coli* is because *E. coli* are an important indicator of faecal contamination of water. A 100 mg of Alum and sodium hypochlorite was also tested against *E. coli* as control. The whole set up was incubated at 37°C for 24 h and the diameter zone of inhibition was recorded in millimeters.

Test tube *in vitro* coagulation and disinfection test using methanol extracts of the plant materials

This was a novel technique meant to further proof phytodisinfecttion activity of the extracts. This technique has not been reported elsewhere preliminary *in vitro* coagulation and disinfection studies was also carried out using the methanol extract from each of the plants. Zero point five (0.5) ml of each of the extract was dropped in 5 ml of Turbid water in a test tube, clearance rate after 10 to 15 minutes residence time was reported visually and a ml of each of the treated water was cultured for total bacterial counts (Yongabi, 2004)

RESULTS AND DISCUSSION

The list of plants used as phytodisinfectants, availability, growth conditions and coagulants in rural cameroon is shown in Table 1. The plants are very widespread across Africa and survive on poor soil as well as different climatic conditions. The result of the physical nature and microbial content of turbid water samples from Bambui, Cameroon before Treatment with Alum and plant materials is presented in Table 2. The total aerobic mesophilic bacterial counts, *E. coli* counts as well as coliform counts were generally high, especially in stagnant, dirty stream water and samples collected from gutters or drainage where microbial counts were too numerous to count (TNTC). Wastewater in Bambui neighbourhood in Bamenda is not properly disposed. There is a possibility the wastewater discharged from homes contaminate surface and ground water use for drinking and domestic chores. A similar observation in Yelwa community in Bauchi, Nigeria was previously reported (Yongabi, 2004). The results in Table 2 served as untreated control all through the experiment. The pH of the turbid water samples was 7.0 using a urinary test trip - Combi 9 test trip while the turbidity which was assessed subjectively using visual observation was noted to be highly turbid with an unpleasant odour for most of the samples except the surface water. This observation has been previously reported for water in a neighbourhood in Bauchi, Nigeria (Yongabi, 2004).

Table 3 shows the results of the coagulative and disinfective effect of *M. oleifera* seeds on turbid water in comparison with aluminum sulphate.

The findings indicated that *M. oleifera* seeds coagulated well above 90% of the particles in the samples leading to a clear supernatant. The coagulation effect was far better in heavily polluted water than in less polluted water and this observation has been previously noted (Yongabi, 2004). Unlike with alum, floc settlement in *Moringa* treated samples was slower when the seeds were directly dispersed into the water but when the seeds were packed in to a muslin sackcloth and dipped into the samples, floc settlement was faster and was as good as with Alum and has been previously observed by Prasad (2009). The coagulative effect of *Moringa* seeds was even better than with Alum and this can be explained with

Table 1. List of plants used as phytodisinfectants and coagulants in rural Cameroon.

Plant material	***Moringa oeifera***	***Aloe barbadensis***	***Jatropha curcas***	***Pleurotus tuberregium***	***Citrus auratifolia***
Family/ Local name botanical name	Horse Radish Zogale (Hausa) *Moringa Oleifera* Lam Moringaceae	*Aloe Vera* Aloes Lilliaceae	Euphorbiaceae Physic Nut Benin Zugo (hausa)	*Pleurotus tuberregium* fr. Singer Mushrooms	Lime
Part used	Seeds				Juice used
Climatic conditions	All types of soil but mostly alluvial sandy soil, hot low lying semi arid areas	All types of soil and survives in different climates	Tropical and sub tropical	Tropical and subtropical areas	Temperate and tropical conditions
Cost of plant material	1 kg of seeds in 5000 fis cfa in Cameroon and 40 naira in Nigeria	Hard to determine as most people grow it	Not determined	1 KG of sclerolium cost 5000 frs in Cameroon	1 KG of limes cost 2000 frs in Cameroon.
Availability and yield	Found all over Cameroon and across Africa. Average seeds per pods and 1 seed purifies a litre of turbid water	Very available across Africa	Very available across Africa	Limited to tropical areas	Yield high in the tropics
Limitation	Treated water must be used in less than 2 days	Impart a slightly bitter taste in water	Seeds, toxic	Cultivation of sclerofia takes long	Impart Specific lime odour and renders water acidic
Remarks from literature survey	Extensive literature in the plant with many tribes aware of its uses. One of the best plant coagulants known. Studies on disinfection limited.	No published work on its use in water purification	Literature exist in its use in water (Yon gabi, 2004; Pritchard et al., 2009)	Yongabi 2004	Little report on used as a coagulant

the fact that *M. oleifera* seeds exhibited strong antimicrobial activity. The raw untreated stagnant water from the gutters or drainage had an initial total bacterial counts Too Numerous to count, which reduced to only 4000 colony forming units per ml when treated with Moringa seeds as opposed to 11000 colony forming units when treated with alum. Alum exhibited a minimal effect on bacterial load of the samples. This may be partly due to the fact that some bacteria attach to the surface of particles that eventually settles with them and as well as the acidic nature of alum might have had some influence on the bacterial counts. To further prove this point, a 100 mgl ml of alum was tested on *E. coli* isolated from the surface turbid water samples and only a traceable inhibition(less than 1 mm) was noticed as opposed to marked inhibition demonstrated by *M. oleifera* (15 mm) and chlorine (17 mm). Meanwhile a 100 mglml of (bulk) methanol extract of *M. oleifera* seeds when tested

Table 2. Physical nature and microbial content of turbid water samples from Bambui, Cameroon before treatment with alum and plant materials.

Type of waste Water sample Counts (Cfulml)	Physical appearance Cfulml	Total aerobic Mesophilic bacteria Cfulml	*Coli* form counts	*Escherichia coli* counts
Dirty water Stagnant in Gutters Lot of suspended Matter	Very dirty and highly turbid with a	TNTC	TNTC	TNTC
Dirty, heavily polluted stream With garbage dumps	Very turbid appearing brownish	800000	52, 000	31000
Kitchen sink samples from Bambui	Dirty, cloudy and appearing very turbid	650000	47,200	6000

Key: CFU----- Colony forming unit per ml, TNTC ------ Too numerous to count.

Table 3. Coagulative and disinfective effect of *Moringa oleifera* seeds and alum on turbid water samples.

Type of water sample	Physical appearance (Arbitrary classification)	Treatment with alum (Aluminum sulphate) (5 g)	Treatment with *Moringa Oleifera* seeds (5 g)
Dirty water	Very dirty	Flocs settled	Flocs formed and settled when directly dispersed in water
Stagnant in gutters	Very turbid with a lot of suspended particles	Clear supernatant Ec = 4500 Cfulml Cf = 3500 Cfulml TBC = 11000 Cfulml	Well settled when held in muslin cloth Supernatant clear Ec = 1500 Cfulml Cf = 5200 Cfulml TBC = 4100 Cfulml
Dirty heavily polluted stream with garbage in Bambui	Very turbid brownish	Flocs formed+ settled Ec = 2375 Cfulml Cf = 47,900 Cfulml TBC = 720,000 Cfulml	Flocs well settled when held in muslin sack cloth, clear supernatant. Ec = 2190 Cfulml Cf = 6100 Cfulml TBC = 73,000 Cfuml
Kitchen sink samples from Bambui	Dirty, cloudy + turbid	Flocs settled Ec = 3152 Cfulml Cf = 5000 Cfulml TBC = 121,000 Cfulml	Flocs settled Supernatant clear Ec = 800 Cfulml Cf = 3215 Cfulml TB = 61000 Cfulml

showed an appreciable inhibition on the same *E. coli*. These results are shown in Table 6.

The coagulative property of *Moringa* seeds could be attributed to a polymeric coagulant earlier reported by Eilert et al. (1978) and Kebreab (2005). The works of Eilert (????) also supports the antibacterial activity of

Table 4. Phytocoagulative and Phytodisinfective effects of *Jatropha curcas* seeds, *Pleurotus tuberregium* sclerotium, *Hibiscus sabdarifa* calyx and Alum on turbid water samples.

Type of water sample	Physical appearance	Alum treatment (control)	Treatment with *Jatropha curcas* seeds	Treatment with *Pleurotus tuberregium* Sclerotium	Treatment with *Hibiscus sabdarifacalyx*
Dirty water stagnant in Gutters	Dirty, Very turbid and suspended particle	Flocs settled clear supernatant Ec = 3150 cfulml Cf = 4982 cfulml TBC = 9300 cfulml	Flocs Formed and settle, clear supernatant Ec = 2254 cfulml Cf = 4458 cfulml TBC = 8115 cfulml	Flocs formed slowly settled supernatant Ec = 3521 cfulml Cf = 4250 cfulml TBC = 8100 cfulml	Flocs formed settled slowly, red pigment extracts, Clear Ec = 2819 cfulml Cf = 13516 cfuml TBC = 52,150 cfuml
Dirty heavily polluted brownish stream at Yelwa, Bauchi colour	Very Turbid	Flocs settled, Very clear supernatant Ec = 18250 cfulml Cf = 39910 cfulml TBC = 61500 cfulml	Flocs settled clear supernatant Ec = 5012 cfulml Cf = 8300 cfulml TBC = 232,000 cfulml	Flocs slowly settled, clear supernatant Ec = 16000 Cf = 3030 TBC = 209000	Flocs formed slowly settled red pigment observed Ec = 3622 cfulml Cf = 4252 cfulml TBC = 52180 cfulml
Kitchen sink sample from yelwa	Dirty cloudy turbid	flocs settled supernatant Ec = 3597cfulml Cf = 3675 cfulml TBC=617000cfulml	Flocs formed + settled, clear supernatant Ec = 1115 cfulml CF = 4500 cfulml TBC108000 cfulml	flocs formed + settled, clear, supernatant Ec = 3526 cfulml Cf = 43000 cfulml TBC = 58100 cfulml	Flocs formed E settled slowly supernatant but red Ec = 440 cfulml Cf = 2351 cfulml TBC = 39725 cfulml

EC---*Escherichia coli*, CF-----coliform, TBC----Total bacterial counts, CFU/ml---Colony forming units per ml.

Moringa oleifera, while Umar Dahot. (1998) and Kebreab (2005) reported the antibacterial action of small protein/peptide against, *E. coli, Klebsiella aerogenes, Klebsiella pneumoniae, S. aureus* and *Bacillus substilis*. The observations in this study corroborates these earlier observations in that *M. oleifera* seeds reduced the total aerobic mesophilic counts drastically and the bacterial isolates listed in their studies are actually aerobic mesophilic bacteria (Amir et al., 2010). A better coagulation and disinfection activity was observed with the methanol extracts of the plants. This corroborates previous views that pure extracts of plant seed powders such as *M. oleifera* possess better coagulant and disinfection activity than the plant materials (Kebreab, 2005; Amir et al., 2010).

The results of the coagulative and disinfective effects of *J. curcas* seeds (Physic nut), *Pleurotus tuberregium* sclerotium, *H. sabdarifa* calyx and alum on turbid water samples are shown in Table 4.

The results generally show that the plant materials possess some disinfective and coagulative effect with *M. oleifera* seeds; *J. curcas* seeds and calyx of *H. sabdarifa* possess both coagulative and disinfective ability. Generally in the experiment, the weight of the plant materials and alum were varied from 0.5 to 5 g per 200 ml turbid water samples. The best coagulative effect was noted between 4 to 5 g plant powders. The result of the experiment was reported using 5 g and previous observed by Yongabi (2004).

When compared to the untreated turbid water samples, all the plant materials except *M. oleifera* (>95%) are between 60 to 90% effective in purifying the water samples used in the study. The coagulative effect of the mushroom sclerotium of *P. tuberregium* was the least of all the plant materials. The first report on the use of this mushroom in water treatment was reported by Yongabi (2004) who observed a similar trend as in this study. The mild effect on the bacterial load of the turbid water may be explained in the fact that extracts of *P. tuberregium* do not possess any antibacterial activity (Tables 5 and 6).

The mild reduction of bacterial load is possibly due to the fact that settled particles in water do have organisms attached to their surfaces. This effect is possibly the case with alum.

Table 5. Preliminary antibacterial activity of methanol extracts of plants and alum on *Escherichia coli* isolated from waste water samples (mm).

Extracts alum (100 mglml)	Sensitivity on *E. coli*
Methanol extract of *Moringa Oleifera* seeds	15
Methanol extract of seed of *Jatropha curcas*	8
Methanol extracts of calyx of *Hibiscus subdariffa*	14
Methanol extracts of sclerotium of *Pleurotus tuberregium*	0
Alum (aluminum sulphate)	6
Sodium hypochlorite	17

Table 6. Disinfection effect of methanol extracts of three plant materials on total bacterial counts of turbid water in CFU/ml.

Treatment	Initial total counts	Total bacterial after
Methanol extracts of Moringa	TNTC	100
Methanol extract of aloe barbadensis gel 85CFU/ML	TNTC	85
Methanol extracts of *Sclerotium* 750CFU/ML of *Pleurotus tuberregium*	TNTC	7500
Sodium hypochlorite 50CFU Control	TNTC	50

TNTC-----Too numerous to count.

The mushroom sclerotium powders coagulated the particles better when placed in muslin sackcloth. This was generally observed for all the plant materials and this is because pulverized plant materials had very small particles that remained in colloidal form. The use of sclerotium of the mushroom *P. tuberregium* to purify turbid water is a significant observation that requires further studies. *P. tuberregium* is an edible mushroom widely found in South and Eastern Nigeria and South Western Cameroon and is a wild mushroom. The sclerotium, which is a dormant stage of the mushroom, can lie in the ground for years and during which it fruits repeatedly at the onset of the rains. In Igbo land in Nigeria as well as in the Bayangi and Bakweri clans in Cameroon the pulverized sclerotium is used in the preparation of a soup delicacy that is well valued by notables in these societies. There are many plants with plants with phytocoagulant and disinfectant ability that needs to be exploited (Table 1).

The seeds of *J. curcas* exhibited a stronger coagulative as well as disinfective effect than Alum. (Tables 4 and 5) but when compared to *M. oleifera* is about 60 to 80% while *Moringa* was above 95%. *J. curcas* is a very common plant with a number of medicinal uses. The root extracts have been used in the treatment of sexually transmitted diseases while the leaf latex has been used as a hemostat (Yongabi, 2004). The use of the powders of the calyx of *H. sabdarifa* has shown both coagulative as well as disinfective effect. It reduced total aerobic bacterial counts, *E. coli* and coliform counts greater than Alum but generates a red pigment extract in the water leaving the water colored. The methanol extract of *H. sabdarifa* calyx demonstrated antibacterial activity (Table 5). The calyx extract of *H. sabdarifa* has been widely used as a local beverage in Northern Nigeria.

Generally, the turbidity of the water samples reduced drastically after treatment with all the plant materials. Alum decreased pH of the turbid water samples from 7.0 to 5 while with the pH of plant materials treated, and untreated water samples was 7.0.

The use of *M. oleifera* seeds as a water coagulant is gaining wide attention (Kebreab, 2005; Pritchard, 2009; Prasad, 2009; Amir et al., 2010). The use of the plant in the treatment of domestic drinking water has been known in Malawi where two grams of the crushed seeds has been used to treat 20 L of water but the use of other plants especially those specified in this study have not been known nor reported elsewhere except in an earlier report of Yongabi (2004).

This study has conclusively indicated that turbid water can be treated considerably with the application of phyodisinfectants and phytocoagulants. The inhibitory effect of *M. oliefera* seeds extracts, *J. curcas* seed extracts and *H. sabdariffa* on *E. coli* isolates as well as the drastic reduction of bacterial load from the turbid water demonstrates that these plants are phytodisinfectants. All the plants studied showed phytocoagulant activity but not phytodisinfectant ability. This research has also shown that there are many plants that need to be screened properly for water treatment (Table 1). The need to exploit the potential of plants may offer cheap, and environment friendly methods of tackling water contamination and may possibly overcome the hazards of using synthetic compounds. From this study, further studies on the actual application of *M. oleifera* seeds in water purification at household or large scale level in

Africa is required.

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