POTENTIALS OF *Calotropis procera* LEAVES FOR WASTEWATER TREATMENT

Shittu, B.O*, Popoola,T.O.S and Taiwo, O.
Department of Microbiology, University of Agriculture. Abeokuta, 110001.Nigeria
*(Corresponding author)

ABSTRACT
Waste water samples from cassava, domestic and textile effluent were treated with leaves of *Calotropis procera* using percolation and dropping methods. Treatment did not significantly reduce colour and taste of treated water, but significant reduction ($p < 0.05$) was observed in turbidity. Treatment also significantly reduced total viable count of waste water by between 31.25 and 100% depending on the method of treatment. Population of coliforms and non-coliforms present in treated water samples also dropped significantly. Although both methods of treatment were effective for treatment of waste water with *Calotropis procera*, the dropping method was more effective in reducing microbial population in waste water.

Keywords: water treatment, *Calotropis procera* leaves, coliforms

INTRODUCTION
Availability of portable drinking water in many developing countries has been a major problem requiring urgent attention. In Nigeria, because of the inadequacy in supply associated with treated water in urban settlements and complete absence of such supply in rural settlements, a greater percentage of the population in big cities, towns and villages depend on untreated water from various sources particularly those from streams, deep wells and other forms of surface / run-off waters. Water supplies from these sources are usually contaminated with organic, inorganic and microbial impurities (Edema *et al.* 2001).

The quality of water from these sources notwithstanding, water from the afore- mentioned sources are often consumed directly and used for other domestic purposes. This is because chemical treatment of water prior to consumption is not within the reach of the rural poor. The risk of waterborne diseases is of paramount importance in this respect. There is therefore, the need for an easy and affordable alternative mode of treating water for domestic uses.

Natural coagulants such as *Moringa stenopetala, Moringa oleifera* (Oluwalana, *et al* 1997), *Hypoestis verticillaria* (Jahn, 1986) have been tested as substitutes for domestic water treatment. Locally, the coagulation attributes *Moringa oleifera* have been found effective in clarifying turbidity of raw water (Oluwalana, *et al.* 1997). Seeds of *Moringa* spp. in particular are important as a primary coagulant which has been used to effectively clarify highly turbid muddy waters and waters of medium and low turbidity which may appear milky and opaque or sometimes yellow or greyish. Often, the action of *Moringa* seeds as a flocculant is almost as fast as that of Alum. Since bacteria in water are generally attached to solid particles, treatment with Moringa seed-powder can leave water clear with 90-99% of the bacteria removed (Sutherland *et. al,* 1990)

Leaf extracts, chopped leaves and latex of the Giant Milk Weed or Sodom apple (*Calotropis procera*) has shown great promise as a nematicide *in vitro* and *in vivo* (Anver and Alam 1992). The plant has also been reported to contain an active non-toxic proteolytic enzyme which is used in the curdling of milk protein in traditional Cheese making in Nigeria (Aworh *et al.* 1994).

The subject of this study is to assess the use of *Calotropis procera* leaves as a natural coagulant for the purification and clarification of raw turbid water and waste water from different sources. The ability of the coagulant leaves of this plant to reduce the population of microorganisms in the water samples is also considered.

MATERIALS AND METHODS.
Collection of water samples
Water samples for analysis were collected in sterilised 2 litre plastic bottles. The samples were collected from tap (public water supply), deep well and stream. Waste water samples from domestic, cassava processing run-off and textile effluent were also collected. Physico-chemical and bacteriological analysis of the water samples were carried out within four days of collection. Sterile distilled water was used for control treatment.

Physico-chemical analysis

pH of the water samples were taken using a pH meter (Jenway, UK). Turbidity was determined with a colorimeter. Colour of the water samples were noted, while human sensory organs was used in determining the odour and taste (except where smell and colour does not make the water sample attractive for tasting). Temperature was determined at the point of collection with a digital thermometer

Bacteriological analysis

Pour plate method was employed in determining the colony forming units (cfu/ ml) on Plate Count Agar (Oxoid), while Multiple tube fermentation technique was used for assessing the Most Probable Number (MPN) counts of the water samples. The results obtained were then compared with FAO standard for the estimation of coliforms per 100ml of water samples. Confirmatory tests were then carried out on positive MPN tubes to confirm the presence of *E. coli*. Pure cultures of isolates obtained by repeated streaking on agar plates were subjected to various tests for characterisation, including, gram staining, catalase coagulation, ability to utilise citrate, sugar utilisation, indole production and other tests as specified in the *Bergey's Manual of Determinative Bacteriology*.

Water Treatment

Leaves obtained from actively growing *Calotropis procera* plants were used for the treatment of water samples. The leaves were thoroughly washed under running tap water and rinsed several time with sterile distilled water. Two methods, the dropping and percolation methods were used; for treating the water samples. In the dropping method, water samples in 100 ml sterile beakers were labelled A to G depending on the source of sample. Previously washed leaves were cut into small pieces (5mm x 5mm), the pieces were dropped into beakers containing the water samples. The beakers were covered with sterile aluminium foil and left on the laboratory bench at room temperature for 24 hours. In the percolation method, washed leaves were used to line sterile funnel, water samples were then aseptically poured into the funnel and allowed to percolate through the leaves into labelled sterile flasks. Other treatments are similar to that of the dropping method. After 24 hours, 5ml samples was taken from the each flask containing treated water samples for analysis.

RESULTS AND DISCUSSION.

The physico-chemical properties of the water samples examined is presented in Table 1. Samples A, B, C and D were obtained from public tap, deep well and Somorin stream and Alabata stream respectively. Sample E was obtained from domestic waste water, while samples F and G were obtained from cassava and textile effluents respectively. Water samples from public water supply, deep well and Obantoko stream were colourless, odourless, tasteless and free of inorganic particles. Water samples from Alabata stream differed from this category with a slight tint of creamy colour. Water samples from domestic waste, cassava and textile effluents were less attractive as they had taste, slightly turbid and characterised by offensive odour.

Table 1: Physical properties of waste water samples

Sample	Odour	Colour	Taste	Presence of particles	Temperature
A	Odourless	Colourless	Tasteless	None	28°C
B	Odourless	Colourless	Tasteless	None	29°C
C	Odourless	Colourless	Tasteless	None	28°C
D	Odourless	Creamy	Tasteless	None	28°C
E	Offensive	Slightly turbid	(Not done)	Cloudy	30°C
F	Offensive	Light yellow	(Not done)	Cloudy	29°C
G	Offensive	Dark green	(Not done)	Particulate	29°C

Key to source of samples

A – Tap water; B- Well water; C – Stream water (Obantoko stream); D – Stream water (Alabata stream) E – Domestic waste water, F – Cassava effluent; G – Textile effluent

Although slight differences were observed in the temperature recorded for the water samples, the differences were not significant ($p > 0.05$).The exceptional high temperature observed on domestic wastewater may be attributed to microbial activities going on the effluent. Except for cassava waste water, pH of water samples did not differ significantly. Marked differences were however observed in the turbidity of the water samples. Cassava and textile waste waters were the most turbid, while water from public tap was the least turbid.

Although both methods of treatment were effective in reducing pH and turbidity of water samples, differences observed in percentage reduction in turbidity was not significant ($p > 0.05$). Percolation method was however more effective and faster than dropping method for pH reduction. (Table 2).The pH of treated water samples improved remarkably and was close to the standard pH recommended for portable water i.e. pH 6.5 - 7.5 (Ademoroti, 1996). The rather low pH value recorded for cassava effluent is likely to be due to the high acidity of cassava liquor as a result of activities of several species of lactic acid bacteria involved in the fermentation of cassava (Oyewole, 2002).

Table 2a: pH of waste water samples before and after treatment with leaves of *Calotropis procera.*

		Treatment			
		Percolation Method		Dropping method	
Samples	Initial pH	pH (after treatment)	% reduction	pH (after treatment)	% reduction
A	8.01	7.50	6.40	6.33	20.90
B	7.61	7.30	4.10	6.10	19.80
C	8.57	7.40	13.70	6.37	25.70
D	7.63	7.18	5.90	6.85	10.20
E	7.05	7.42	5.20	6.40	9.20
F	3.10	3.22	NA*	3.10	0.00
G	8.22	7.68	6.60	6.70	18.50

A – Tap water; B- Well water; C – Stream water (Obantoko stream); D – Stream water (Alabata stream)E – Domestic waste water, F – Cassava effluent; G – Textile effluent
* NA= Not applicable, pH value increased with treatment

Table 2b: Turbidity of waste water samples before and after treatment with leaves of *Calotropis procera.*

		Treatment			
		Percolation Method		Dropping method	
Samples	Initial Turbidity	turbidity (after treatment	% reduction	turbidity (after treatment	% reduction
A	0.08	0.03	62.50	0.08	0.00
B	0.14	0.03	78.60	0.08	42.90
C	0.13	0.09	30.80	0.10	23.10
D	0.50	0.04	92.00	0.15	70.00
E	0.45	0.20	55.60	0.01	97.80
F	0.99	0.26	73.70	0.40	59.60
G	1.00	0.59	41.00	0.35	65.00

A – Tap water; B- Well water; C – Stream water (Obantoko stream); D – Stream water (Alabata stream) E – Domestic waste water, F – Cassava effluent; G – Textile effluent

Dropping method of water treatment with leaves of *Calotropis procera* was more effective in reducing microbial load in waste water (Table 3). For all the water samples, the counts of microorganisms present in all the water samples exceeded the standard limit. With treatment, the total viable count and Most Probable Number (MPN) were drastically reduced. Over 80% reduction was observed in some cases even on domestic, cassava and textile waste water with percolation method and 100% reduction in some cases for water treated using the dropping method. The dropping method and percolation methods differed significantly ($p < 0.05$) in their ability to effectively reduce microbial load of water samples. The dropping method was more effective in reducing the population of microorganisms in the water samples.

Table 3: Total viable count and Most Probable Number (MPN) of coliforms /100ml of water samples

Sample	Total Viable Count					Most probable No / 100ml water sample				
		Percolation method		Dropping method			Percolation		Dropping	
	Untreated	Treated	% reduction	Treated	% reductio n	Untreated	Treated	% redu-ction	Treated	% redu-ction
A	$1.6x10^3$	$1.4x10^3$	12.50	$4.0x10^2$	75.00	16.00	11.00	31.25	0.00	100.0
B	$14.0x10^3$	$8.4x10^3$	40.00	$7.0x10^2$	95.00	16.00	9.40	41.25	0.00	100.0
C	$15.0x10^3$	$10.0x10^3$	33.33	$25x10^2$	83.30	44.00	20.00	54.55	3.60	91.80
D	$10.0x10^3$	$5.0x10^3$	50.00	$20x10^2$	80.00	24.00	7.20	70.00	0.00	100.0
E	$17.0x10^3$	$8.5x10^3$	50.00	$29x10^2$	82.90	43.00	15.00	62.12	11.00	74.40
F	$7.0x10^3$	$3.5x10^3$	50.00	$9.0x10^2$	87.10	9.30	6.00	35.48	3.00	67.70
G	$12.0x10^3$	$6.7x10^3$	44.20	$14x10^2$	88.30	19.00	11.00	42.11	7.30	61.67

A – Tap water; B- Well water; C – Stream water (Obantoko stream); D – Stream water (Alabata stream) E – Domestic waste water, F – Cassava effluent; G – Textile effluent

Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and *Streptococcus faecalis* were the major bacterial species encountered in most of the waste water treated. *Lactobacillus plantarum, Leuconostoc* sp, *Bacillus substilis* and *Saccharomyces* spp were isolated from cassava effluent. Total viable counts for all the water samples were generally high, in most cases, occurring in excess of the permissible limit. This makes them unsuitable for drinking and other uses. The counts of these organisms in tap water were comparatively low. (Table 4).

The presence of *Escherichia coli* and *S. faecalis* in water samples is an indication of faecal contamination. The high counts of these Enterobacteriaceae in Alabata and Obantoko stream water samples is an indication of poor hygiene practices associated with local communities as they defecate in streams along its flow.

Table 4: Microorganisms isolated from waste water samples

Organisms	Water samples						
	A	B	C	D	E	F	G
Escherichia coli,	-	+	++	++	++	+	-
Staphylococcus aureus,	-	+	+	+	+	-	+
Pseudomonas aeruginosa	-	-	-	+	+	-	+
Streptococcus faecalis	-	-	+	+	+	-	-
Lactobacillus plantarum,	-	-	+	+	-	++	-
Leuconostoc sp,	-	-	+	+	+	++	-
Bacillus substilis	-	+	+	-	-	++	-
Saccharomyces spp	-	-	-	-	-	++	-
Others (including Protozoa, algae, etc)	-	-	+	++	-	-	+

A – Tap water; B- Well water; C – Stream water (Obantoko stream); D – Stream water (Alabata stream), E – Domestic waste water, F – Cassava effluent; G – Textile effluent
+ =Present; ++ = Present in very high count; - = absent

The ability of *Calotropis procera* leaves to reduce total viable count of microorganisms in water samples can be attributed to some active ingredients it contains. Chaudhuri *et al.* (1985), identified mudarin, yellow bitter acids and calotropin as present in the leaves of *Calotropis procera*. Most of these compounds particularly calotropin have been reported to have anti-bacterial properties (Aworh *et al.* 1994)

The advantage of dropping method over percolation method in reducing the microbial load in water samples could be attributed to the longer contact time extracts from the leaves had with the water samples. This enables the active ingredient in the leaves to diffuse freely into the water samples. Fortunately too, there seems to be no danger of toxicity of extracts from the leaves as it is already being widely used locally in milk and cheese processing (Ogundiwin and Oke, 1983). The coagulating properties of the leaves might have also helped in trapping the microorganisms, hence the ability of extracts from the leaves to reduce population of microorganisms in waste water.

Use of natural materials of plant origin for the clarification of turbid water has been used successfully for the primary processing of water for domestic uses (Aririatu *et al.* 1999, Sutherland, 1990; Sutherland *et al,* 1990; Holmes *et al.* 1993; and Travis *et al* 1993). Results of this experiment have shown that leaves of *Calotropis procera* have the potentials for the treatment of water samples. Although, the treated water samples as observed in this study were not microbiologically safe, drastic reduction in turbidity, pH and microbial load of such water makes it attractive for other domestic uses.

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