

Studies on the Efficacy of *Bridelia Ferruginea* Benth. Bark Extract in Reducing the Coliform Load and BOD of Domestic Wastewater

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ABSTRACT

The efficacy of *Bridelia ferruginea* bark extract in reducing the coliform load and BOD of Wastewater was investigated. Phytochemical screening and chromatographic techniques revealed the bark to contain five major compounds; polyphenols, steroids, Saponins, Tannins terpenoids and alkaloids. Comparative studies in the reduction of coliform load using varying concentrations (0.5% w/v, 1.0% w/v, 2.5% w/v, and 5.0% w/v) with Alum and Ferric chloride showed that the bark extract was effective. The optimum dose achieved was 2.5% w/v with a minimum of 24 hours contact time. The coliform loads were reduced by 63% after 24 hours when the extract was used whereas Ferric chloride achieved 64% reductions and Alum achieved 68% reduction under similar conditions.

Comparative studies of Biological Oxygen Demand (BOD) removal from the wastewater using varying concentrations (1% w/v, 5% w/v) with Alum and Ferric chloride showed that the bark extract achieved 100%. The feasibility of using the bark extract as an additional coagulant is therefore discussed.

Key Words: *Bridelia ferruginea*; Coliform, Biological Oxygen Demand (BOD), wastewater Treatment.

INTRODUCTION

Water is one of the most important natural resources.(1) In fact, life on earth could not go on if deprived of this amazing liquid and evolution could never have taken place without it.(2) Also, water is among the essential requisites that nature provides to sustain life for plants, animals and humans and the total quantity of fresh water on earth could satisfy all the needs of the human population if it were evenly distributed and accessible(3). Water is an important resource being used in a variety of ways at many different levels, which produces social, spatial and organizational problems.

There is no end to which water can be described which led to (4) referring to water as having the peculiar quality of being an inexhaustible natural resource which, nevertheless is in short supply.

Municipal water demand can commonly be classified according to the nature of the user. The ordinary classification are domestic, commercial/ industrial and public use. Generally, water can be used for drinking, food preparation and cooking, cleaning and washing and personal hygiene, vegetable garden watering, stock watering and other uses including waste disposal and industrial uses (5) From the earliest times including the period of industrial revolution, streams, lakes, lagoons and seas have been a natural place to discharge waste

leadings to pollution of these water resources. It may be in form of solid, liquid or gases; all these adversely affect the environment (6).

Water quality requirement vary according to the proposed use of the water. Turbidity which is one of the character of water considered for its portability may come from erosion of clay banks, domestic and also from industrial wastes. Water is classified as polluted if it is clearly dirty in appearance and has an unpleasant taste.

It may contain organic matter, which makes it unpleasant for drinking and other uses. It may also be altered in composition or condition, directly or indirectly as a result of man's activities (i.e. wastes) so that it becomes less suitable for anyone or all of the uses it could be put. (7)

The coliform group has gained widespread acceptance among water analysts as the best measure of faecal contamination (8). The "coliform bacteria" belong to the family *Enterobacteriaceae* and are gram-negative rods measuring some 2-5 diameters by 0.4 micrometer. The coliform group includes all the aerobic and facultative anaerobes, non-spore forming rod-shaped bacteria, which ferments lactose with gas formation within 48 hours at 37°C. Coliforms are generally present in large numbers in human excrement and can be detected in numbers as small as one in 100ml of water. They are commensals and constitute part of the intestinal microflora and are potentially pathogenic elsewhere in the body where they produce pyogenic infections in children, elderly people and those debilitated by other illnesses (9). Thus, the presence of coliforms in water sample indicate that intestinal pathogens may be present although perhaps in a fewer number. Standard of microbiological purity for portable waters are normally quoted as total coliform or *Escherichia coli* concentration per 100ml of sample water. (10)

Water pollution already is a serious problem in the majority of the developing countries. A high proportion of domestic and industrial effluents are untreated and discharged directly to water courses, irrigation canals, and drainage ditches. Allowed to continue, this increased pollution will reduce the amount of water available for use in the future (11). While the physical availability of water to each country is unique and usually constant, demand for water will continue to increase. The problem is how to balance demand and supply.

Producing high quality reclaimed water from wastewater treatment plants requires a paradigm shift for operators. Communities must explore the construction of tertiary treatment trains on existing wastewater treatment processes, additional transmission lines and constructions and development of reclaimed water discharge alternatives. Planners also must develop wastewater master plans that include reuse alternatives and treatment levels appropriate to each beneficial reuse. Consumers also will need assurance that the reclaimed water is safe and they are adequately protected from deleterious substances. The initial success of reuse projects will be sustained only if the public perceives that the reuse of wastewater is healthy and necessary (11).

There are very many methods of domestic wastewater treatment, which follows the same trend. Chemical precipitation, an early wastewater treatment method, involved the addition of early wastewater treatment method, involved the addition of lime, iron sulphate and other coagulants to cause organic and inorganic solids to settle out of wastewater (12).

Further treatment (tertiary) of a biological treated effluent is carried out to remove BOD₅, bacteria, suspended solids, specific toxic compounds or nutrients to enable the final effluent to comply with a standard more stringent than 20:30 before discharge. This work intend to study the use of *Bridelia feruginea* benth bark extract as a coagulating agent in wastewater treatment.

Bridelia ferruginea benth bark is the most common savannah *Bridelia*. It is usually a gnarled shrub which sometimes reaches the sizes of a tree in suitable condition. Its common names are Kizni, Kirni (Hausa); Marehi (Fulani), Iralodan (Yoruba), Ola (Igbo), Kensange abia (Boki) (13). However, the flocculating value of the plant has recently attracted the attention of non-governmental organization (NGO) and research centres in developing countries. Recently, the bark extract of the plant has been used for the coagulations of milk and also lime juice for the formulation of a traditional gargle “Ogun efu” (14). A decoction of the leaves is used to treat diabetes. It is also used as a purgative and a vermifuge (Cimanga *et al.*, 1999). (15). The effect of the stem bark and leaf extracts of *Bridelia ferruginea* on skeletal muscles has been studied. (16)

The bark extract of the plant has demonstrated antimicrobial activity against microorganisms commonly known to cause enteric and secondary upper respiratory tract infections.(17) Also, the ability of the bark extract in the reduction of total bacterial count, significant sedimentation of total solids and clarification of river water has been reported. (18) The reduction of total bacterial count, significant sedimentation of total solids and clarification of domestic wastewater using the bark extract has also been reported (19).

Further research into it’s efficacy in the reduction of coliform load and BOD removal in wastewater treatment is desirable. With rapid population growth and the accompanying urbanization, there is bound to be greater demand for water for public utilities, which in turn, may require more direct water re-use or intensification of indirect re-use (20). In these circumstances, removal of harmful coliform from wastewater discharged into public water course becomes extremely important.

Cost benefits ratios will be increasingly rewarding for water reuse and recycle research throughout this millennium. In this study, the effect of *Bridelia* on coliform load in wastewater was determined by the standard plate count. The Biological Oxygen Demand was also determined by the standard dilution technique.

MATERIALS AND METHODS

PLANT MATERIAL

Bridelia ferruginea bark was collected from the tree of *Bridelia ferruginea* benth family Euphorbiaceae from the residential quarters of the University of Ilorin, Nigeria. A voucher sample was deposited at the Biological Sciences herbarium of the University.

PREPARATION OF THE BARK EXTRACT

The bark pieces were cut into small pieces and dried in an oven at 40⁰C for 48 hours (Gallenkamp Oven Bs Size two). The dried pieces were then pulverized using the laboratory mill (Christy and Norris limited, machine type 8) and the powder obtained were stored as stock from which appropriate amounts were taken for experiment.

Extraction: Two hundred grams of the powdered bark were extracted with solvent combination of water and ethanol in the volume ratio of 1:2 at room temperature for 48 hours. (2 days).

The suspension was then decanted and filtered using sterile Whatsmann Paper No. 1. The filterate was concentrated to dryness at 45⁰C in a rotary evaporator. The residue obtained served as the bark extract (21).

PHYTOCHEMICAL ANALYSIS

The screening procedures were adapted from those of (22). The extract were screened for the presence of Alkaloids, Tannins, Terpenoids, Glycosides, Flavonoids, Saponins, Anthraquinones and steroids.

1. **ALKALOIDS:** 1.5ml of 10% HCl was added to about 5ml of the extracts in a test tube. The mixture was heated for 20 minutes. It was cooled and filtered 1ml of the filterate was tested with few drops (5 drops) of Mayers and Dragendorff’s reagents. A whitish yellow and reddish precipitate observed in the extract tested as indication of the presence of alkaloids in the extracts.

2. **TANNINS:** 3 drops of 5% ferric chloride was added to 1ml of the extract. A greenish black precipitate observed in the extract was taken as indication of the presence of tannins in the extract.
3. **GLYCOSIDES:** 10ml of 50% HCl was added to 2ml of the extracts in a test tube. The mixture was heated in boiling water for 30minutes. 5ml of fehling's solution was added and the mixture was boiled for 5 minutes. A brick-red precipitate observed in the extract tested as indication of the presence of glycosides in the extract.
4. **SAPONINS:** Frothing test: 2ml of the extract in a test tube was vigorously shaken for 2 minutes. The frothing which persisted for 5 minutes and when warmed on water bath was taken as indication of the presence of saponin in the extract.
5. **STEROIDS:** Liebermann's Burchard test: 1ml of the extract was dissolved in 0.5ml of acetic anhydride and cooled well in ice. This was mixed with 0.5ml of chloroform and 1ml of concentrated H₂SO₄ was then carefully added by means of a pipette. At the separating level of the two liquids, a reddish-brown ring was formed, as indication of the presence of steroids.
6. **TERPENOIDS:** Ketonic terpenoids were located by dissolving 0.5g of 2,4- dinitrophenylhydrazine in 100ml of 2M HCl. 1ml of the mixture was added to 2ml of the extract. A yellow-orange colouration was observed as indication of the presence of a terpenoid.
7. **FLAVONOIDS:** Shibata's reaction: 3ml of extract was warmed with three pieces of magnesium turning's and mixed with 3 drops of concentrated HCl; An orange pink colouration was taken as indication of the presence of flavonoids.
8. **ANTHRA QUINONES:** Borntrager's test: 5ml of the extract was dried and shaken with 3ml petroleum ether. The filtrate was added to 2ml of a 25% ammonia solution. The mixture was shaken and a red colouration observed was taken as indication of the presence of anthraquinone.

THIN LAYER CHROMATOGRAPHY

The water and ethanol crude extract (1:2) was spotted and examined using TLC precoated plates (silica gel Gf 254, 0.25mm Merck W. (Germany)). These were developed using a mixture of petroleum ether and diethylether (3:1). After the development, the chromatogram was dried and viewed under UV lamp at 366nm and 254nm respectively. Five components were observed and their corresponding R_f values were noted.

PREPARATIVE THIN LAYER CHROMATOGRAPHY (PTLC)

This was used to isolate and purify the phytochemicals in the extract. Glass plates (20 x 20cm) were coated (0.5mm) with silica gel, (Gf 254, 60mesh) used according to the method described by (23). The solvent system was petroleum ether –diethylether (3:1). After development and viewing under UV lamp: The observed bands were Scraped having correlated their respective R_f values with the TLC R_f values before elution was done.

EFFECT OF BRIDELIA ON COLIFORM LOAD

Coliform load of the wastewater was estimated using the plate count method (24). One milliliter of a 10⁻⁴ dilution was plated on Eosin methylene Blue agar (oxid) and incubated at 37⁰C for 48 hours. At 0,24, 48 and 96 hours, 1ml was carefully measured from the treated portion of the wastewater (Varying concentrations of the coagulants added to wastewater sample) serially diluted and enumerated for total coliform on the same media. The same procedure was followed for the Alum and Ferric chloride treated water. Experiments were conducted in duplicates.

EFFECT OF BRIDELIA ON BOD

Preparation of Dilution water: Before used, the distilled water in cotton-plugged bottles was stirred long enough to permit it to become saturated with D.O (Dissolved Oxygen). The desired volume of distilled water was then placed in suitable bottle and 1ml each of phosphate buffer, Magnesium sulfate, Calcium chloride and Ferric chloride solutions were added for each litre of water (24). Dilution technique: Several dilutions of the prepared samples were made so as to obtain the required depletions. The following dilutions were made; 2% and 3.0% in the course of this research work, which falls within the acceptable, range of 1-5% dilutions for raw and settled sewage as recommended by (24).

Standard dilution water was carefully siphon into a graduated cylinder of 1,000 to 2,000ml capacity, filling the cylinder half full without entrainment of air. Carefully mixed samples and coagulants were added to make the desired dilution and diluted to the appropriate level with dilution water. The mixture was mixed well with a plunger –types mixing rod avoiding entrainment of air. The mixed dilutions were then siphon into two BOD bottles (300mls); one for incubation and the other for determination of the initial DO in the mixture; stopper tightly and incubated for 5 days at 20⁰C. The BOD bottles should be water-sealed by inversion in a tray of water in the incubator or by the use of a special water-seal bottle. Succeeding dilutions of lower concentrations were prepared in the same manner.

Titration: 20ml of the liquid supernatant were dispensed into a conical flask and starch solutions were added to it, giving a blue-black colouration. 0.025N Sodium sulphate solution was titrated with the blue-black solution to the starch –iodide end point, which is colourless.

RESULTS

The results of the chromatographic techniques and phytochemical screening is represented in Table 1. The result revealed that alkaloids, terpenoids, saponins, steroids, and tannins were present in the extract. Anthraquinone, flavonoids and glycosides were not found in the extract.

This layer and preparative thin layer chromatographic techniques were used to isolate different phyto-components from the crude extract. The crude extract developed with petroleum ether-diethyl ether (1:3) revealed four components which were thoroughly purified using PTLC to give the phytochemicals as shown in Table 1.

The results of the effect of the coagulants on the coliform load of the wastewater sample is represented in Table 2. Result showed that there was significant reduction in coliform load at any particular dosage of the coagulant for the processed samples compared to the control (raw wastewater sample). However, after a holding time of 96 hours, a higher percentage reduction was achieved with 2.5% w/v dose of bark extract compared to Alum and Ferric chloride. The greater the amount of the coagulants, the more the acidity and the more the reduction in coliform load until an optimum dose is achieved.

The results of the effectiveness of the coagulants in the reduction of Biological Oxygen Demand (BOD) is shown in Table 3. It was revealed that there was appreciable reduction in BOD₅ of the test wastewater sample at any concentration of coagulants used. *Bridelia* bark extract exhibited the best performance regarding BOD₅ removal. It was also found to be more effective than others at any dosage. *Bridelia* bark extract achieved total depletion (100% reduction) of BOD₅ in the wastewater sample.

DISCUSSION

During the past decade, there has been growing concern that the world is moving towards a water crisis. Water is increasingly scarce in dry climate regions (for example, Africa and South Asia), and there are major political implications for the scarcity of water in some regions (for example, the middle East) (25). Issues of both water quantity and quality are of concern. The reuse of wastewater is one of the main options

being considered as a new source of water in regions where water is scarce. The standards required for the safe use of wastewater and the amount and type of wastewater treatment needed are contentious. The cost of treating wastewater to conform to high microbiological standards can be so prohibitive that in many developing countries, the use of untreated wastewater is effectively unregulated (25).

It has been shown in many studies that domestic wastewater usually contain various pathogenic organisms. Some studies revealed that species such as the agents causing typhoid fever, bacillary dysentery, amoebic dysentery, ascaris and other protozoan and helminthic diseases were isolated from wastewater. Other studies have indicated the detection of major enteric viruses in raw domestic wastewater (Ademoroti, 1980). Detection of total coliforms in any kind of water implies the possible presence of pathogenic microorganisms in water and indicates that faecal pollution of the water has occurred. Removal of total coliforms from water, therefore safeguards public health risks (26).

In this work, the coagulating properties of *Bridelia ferruginea* benth bark have been established and found to compare favourably with other coagulants such as Ferric chloride and Alum. The result of the chromatographic techniques and phytochemical screening revealed the bark extract to contain tannins and alkaloids as its major bioactive constituents while other active agents includes steroids, terpenoids and saponins (Table 1). This observation agrees with the result obtained by (27).

Coagulating properties of *Bridelia ferruginea* benth bark have been established and found to compare favourably with other coagulants such as Ferric chloride and Alum in the reduction of total bacteria count of river water (18). In this study, the ability of the bark extract to reduce the coliform load of wastewater can be explained in two ways; one, it made the wastewater acidic, thereby assisting in the removal of sizeable percentage of total coliform load. Also, the cations forms complexes with the enteric bacteria, which caused further reduction in the total coliform population. (20) The percentage removal of the enteric bacteria was appreciable even at lower doses of the coagulants, (Table 2). This result is in consonance with the work done by (20) who explained the removal of coliform from sewage through chemical coagulation and flocculation. The effectiveness of the coagulants in Biological Oxygen Demand (BOD) removal was revealed in this study. *Bridelia* bark extract showed outstanding activity of 100% total depletion of Biological Oxygen Demand (BOD) of the wastewater sample at any concentration compared to Ferric chloride and Alum (Table 3).

These encouraging findings further support the recommendation of *Bridelia ferruginea* bark extract as an additional coagulant because it is cheap, readily available and serves as an easy means of reuse alternatives of wastewater, especially in remote areas. Driven by high water demand, research and technology for wastewater treatment and monitoring will continue to break the barriers of affordability, ease of operation, safety and efficiency (11).

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Table 1. Chromatographic Separation with Rf Values and Phylochemical Screening of the Extract of the Bark of *Bridelia Ferruginea*.

Components	Rf value	Flavonoids	Alkaloids	Tannins	Glycosides	Saponins	Steroids	Anthraquinones	Terpenoids
1	0.30	-	-	++	-	-	-	-	-
2.	0.39	-	-	-	-	-	-	-	+
3.	0.46	-	++		-	-	-	-	-
4.	0.56	-	-	-	-	-	+	-	-
5.	0.85	-	-	-	-	+	-	-	-

Key: ++ = Highly Present,

+ = Present

- = Absent

Table 2. Effect of Coagulants on the Reduction of Coliform Load of Wastewater Sample.

Contact time (hrs)	Percentage concentration (% w/v)	Percentage Reduction %		
		<i>Bridelia ferruginea</i>	Ferric Chloride	Alum.
Zero	Raw sample	-	-	-
24	Raw sample	6.25	6.25	6.25
	0.5	56.25	55.21	57.29
	1.0	58.33	59.34	63.54
	2.5	62.50	63.54	67.71
	5.0	41.67	43.75	46.88
48	Raw sample	14.58	14.58	14.58
	0.5	62.50	63.54	64.58
	1.0	62.50	62.50	68.75

	2.5	67.71	68.75	70.83
	5.0	46.88	47.92	51.04
72	Raw sample	36.46	36.46	36.46
	0.5	70.83	70.83	73.96
	1.0	73.96	75.00	77.08
	2.5	77.08	77.08	79.17
	5.0	53.13	55.21	58.33
96	Raw sample	45.83	45.83	45.83
	0.5	73.96	75.00	77.08
	1.0	76.04	77.08	79.17
	2.5	78.13	78.13	79.17
	5.0	54.17	57.29	60.42

Table 3. Effects on Biological Oxygen Demand (BOD) Using Varying Concentrations of the Coagulants in Wastewater Sample.

Concentrations of coagulants/ sample	Percentage dilution (%)	Sample volume (ml)	DO _{zero} value	D0 ₅ value	Drop	BOD ₅
Control (wastewater alone)	2	6	8.8	8.2	0.6	30
	3	9	8.8	8.0	0.8	26.6
1% w/v Alum + Wastewater	2	3mls +3mls	8.9	8.7	0.2	10.0
	3	6mls + 3mls	8.0	7.5	0.5	16.6
5% w/v Alum + wastewater	2	3mls + 3mls	8.3	7.9	0.4	20.0
	3	6mls + 3mls	8.7	8.0	0.7	23.3
1% w/v FeCl ₃ + wastewater	2	3mls + 3mls	8.7	8.1	0.6	30.0
	3	6mls + 3mls	8.5	8.0	0.5	16.6
5% w/v FeCl ₃ + wastewater	2	3mls+ 3mls	9.5	8.2	1.3	65.0
	3	6mls + 3mls	14.7	11.8	2.9	96.6
1% w/v EXTRACT + wastewater	2	3mls + 3mls	7.3	*	*	*
	3	6mls + 3mls	6.8	*	*	*
5% w/v Extract + wastewater	2	3mls +3mls	5.8	*	*	*
	3	6mls +3mls	6.2	*	*	*

*Depleted

Note: For 2% v/v Dilution → 3mls of wastewater + 3mls of coagulant in solution.

For 3% v/v Dilution → 6mls of Wastewater + 3mls of coagulant in solution.

