Study on the Germicidal Effect of Bitter Bark Tree (*Sacoglottis gabonensis*) on Water Purification

Abstract

The germicidal effect of bitter bark tree was the focus of the study. It aimed at determining the inhibitory effect of the plant bark extract in reducing the microbial load in water, and to evaluate its suitability as natural alternative to chlorination, ozonation, UV treatment, micro-filtration and reverse osmosis. It is evident that our natural sources of water contains high amount of microbes, some of which are viral, bacteria or fungi in nature. The extraction of the plant bark was done with water and alcohol respectively. Water from different sources were collected and mixed together before treatment. The extraction was done in three stages and at different concentrations of the bark extracts, namely 0.1 g, 0.2 g and 0.3 g respectively. Extraction was done using cold and hot water for aqueous extracts, while ethanol was used for alcoholic extracts. Two media – Eosin methylene blue and MacConkey agar were employed for the total coliform count. The result shows that there was no trace of coliforms in the water after treatment with alcoholic extract at all concentrations. However, the hot aqueous extract was more effective than the cold extract within the operating concentrations. Thus, the process is temperature and concentration dependent as increase in both resulted in elimination of the coliforms. However, the organoleptic properties of the water such as taste and smell were preserved. Thus, there is a slight change in the colour of the water which was taken care of with activated charcoal. The work therefore shows that *Sacoglottis gabonensis* bark extract contains anti-microbial agents, and hence can serve as a suitable preservative for water both for domestic and industrial purposes.

Keywords: *Sacoglottis gabonensis*, water. aqueous extract, alcoholic extract, coliforms

1. INTRODUCTION

Water is a universal pure natural resource. It is a universal solvent for most solutes and its use is found both in domestic and industrial purposes. It contains oxygen and hydrogen, and both elements are essential in all living cells. The average volume of this essential component in all living things varies according to types, mass and age. In humans, it is about 57-65%. However, human activities such as oil spills, dumping of refuse, channeling of domestic sewage and industrial wastes into or near water bodies which are flushed into the water during the rains has promoted the growth of algae, hyacinth and wide species of microbes such as coliforms.

Examination of water to ascertain its usefulness for both homes and industries shows that most available sources of water contains some or almost all classes of microbes such as Coliforms,

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Giardia Lambia, Cryptosporidium and Hepatitis A. This trait necessitated the search for efficient means of sanitizing the resource. However, a number of advanced methods have been employed in a bid to sanitize drinking water. Some of those methods includes reverse osmosis, ozonation, UV Irradiation micro-filtration and chlorination. Studies confirmed that antimicrobial activities of plants have been accredited to existence of phytochemicals in specific part of plants. Adeyemi *et al.* [1] in a reviewed various scholarly published articles of over 200 relevant literatures sought from science direct, Google scholar and web science. The researchers included, in the scope, the history of the plants, types, mechanism of action, the methodology of extraction as well as the application of the phytobiotics of the plants under review. Microsoft Excel was used in data computation. Their findings indicate that plants possess multifaceted components which can be sustainably developed into products for water treatment. Thus, their findings revealed that both seed (24.58%), flower (20.75%), leaf (16.98%), resin (1.89%), bark (1.89%) and tuber (1.89%) is useful tool for water treatment. This suggests vast exploration on the use of phytobiotics in water treatment since the ratio of antimicrobial activities to water treatment application is (63.63%) as against (36.37%). Among evidences of plant phytoactivity against microbes that of [2] on the efficacy of aqueous plants extract of *Acacia niloptica*, *Acacia segal*, *Acacia tortilis*, *Acacia etbiaca* and *Albiz anthelmintica* for water treatment capacity. A report indicates a notable effect towards inhibiting the proliferation and eradication of microbes. Studies of *Moringa oleifera*, *Jatropha curcas* and quar gum on water has also been investigated for their water treatment potential and reduction in turbidity of the treated water were observed [3]. In addition to the above literatures, antimicrobial activities of eight mixtures of different extracts studied for their application to remove coliforms isolated in grey water and the use of thyme oil to treat *E. coli* in water recorded higher inactivation of the *E. coli* when compared to ozonation and chlorination [4].

Available data on cytoprotection *in vivo* and *in vitro* studies have shown presence of bioactives in significant amount in both living things like human and rat and in nonliving components such as palm wine and oils [5]. In a related study by [6], herbal disinfection of water was carried out using *Oscimum sanctum*. Aqueous and alcoholic extracts of 50 g of the dried leave were carried out using 100 ml of the solvents. Antimicrobial activities of alcoholic and aqueous extracts were studied on different concentration and time intervals. Results showed that alcoholic extract exhibited complete inhibition at 1.138 mg/ml concentration at the 4^{th} hour, while the aqueous extract showed complete inhibition at 113.8 gm/ml at 5^{th} hour. The most probable number (MPN) test were carried out using alcoholic and aqueous extracts, and result showed that there were 1600 coliforms per 100ml of the untreated water, 500 coliforms per 100 ml of sample treated with 113.8 mg/ml of aqueous extract and 240 coliforms per 100 ml of sample treated with 1.138 mg/ml of alcoholic extract. [7] investigated the comparative effect of *S. gabonensis bark* extract (0.25%) and *A. boonei* (0.50%) on the kinetics of saccharomyces cerevisiae isolated from palm wine. The rate constant for *S. gabonensis*, *A. boonei* and the control were $1.1x10^{-4}$ mol

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Comment [a6]: These results should be added in results and discussion section as in introduction you need to highlight the background and scope of the study only.

$^{-1}$ s^{-1}, 1.7 x 10^{-4} $m^{-1}s^{-1}$ and 2.79 x 10^{-4} $mol^{-1}s^{-1}$ respectively. However, yeast growth was enhanced in the ratio of 0.43: 0.76: 0.88 respectively.

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Bitter bark tree botanically known as *Sacoglottis gabonensis*, cherry mahogany a member of the Humiriaceae family is a rain forest tree of southern Nigeria, native to South America and West Africa has been commonly used in these rural communities as an additive to palm wine. Practitioners believe that it prolongs the shelf life of palm wine thus suggesting antioxidant properties. Available literatures on the efficiency of *Sacoglottis gabonensis* include the studies of [8] on the disposition of acetaminophen in human volunteers by *Sacoglottis gabonensis* treated palm wine. The effect of the plant bark extract on anticoagulant properties and serum levels of acetyl salicyclic acid and acetaminophen by [9] has been established. In addition to the duo, [10] tried to ascertain whether or not the antioxidant properties observed in biological system can also be obtained in non-living systems. The levels of typical peroxidation products (lipid hydroperoxide, malondialdehyde and malonnaldehyde) were determined in the bark extract treated vegetable oils. (Groundnut, palm oil and soya oil) stored for a long period of time in an environment conducive for lipid peroxidation. That of [11] on the enhanced effect of the plant bark extract against membrane lipid peroxidation induced by 2,4-dinitrophenyl hydrazine. Treatment with the plant bark extract significantly reduced the deterioration ($P < 0.5$) during storage) and formation of lipid hydroperoxide, the first major product of lipid peroxidation in all oils stored over a period of three months as compared to the bark extract free control. Thus, their findings suggest that *S. gabonensis* bark extract possess antioxidant properties against lipid peroxidation. Among other related studies on *S. gabonensis* include that of [12] on the influence of *S. gabonensis* bark extract and its isolate bergenin on the metabolic and haematological side effect of 2,4-dinitrophenyl hydrazine-induced tissue damage on the brain and blood of male weaving rats. 2,4-dinitrophenyl hydrazine was mixed in their drinking water for three days to induce lipid peroxidation. The blood, brain and red blood cells of the rats were analyzed for glucose level after three hours of for selected key indices of oxidative stress. Haematological count, red blood cell (RBC), packed cell volume (PCV) and white blood cell (WBC) were examined. Result obtained showed that the plant extract inhibited the glucose depleting action of 2,4-DNPH and ethanol on haemoglobin (HB) count fraction, PCV, RBC. It also inhibited the proliferation of WBC. Thus, the same effect was observed for bergenin. They therefore believed that bergenin was responsible for the antioxidant properties of the plant back against the oxidants. In a similar study, [5] on the effect on *S. gabonensis* bark extract on shelf life of palm wine. Five levels of the concentration in the palm wine ranging from 0.29 gdm^3 to 0.23 gdm^3 were prepared alongside with the control. The bottles were cooked and posturized at 80* for 20 min and stored at room temperature. The pH was measured and acidity was carried *via* titration with standard solution of NaOH. It was found that, addition of the plant bark extract in palm wine lowered the rate of fermentation by inhibiting the growth of acetobacter species found in palm wine. These species acts on sugars in palm wine. Thus, converting ethanol into ethanoic acid, and hence the preservative effect. There is need to study this natural products in a view to

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establish its role in cytotoxic disorders, antimicrobial and antioxidant activities. However, there is no available literature on the antimicrobial action of *Sacoglottis gabonensis* bark extract in water. The work therefore aims at finding out if the plant bark extract can reduce the microbial load in water, hence informing its use as a cost effective means of water purification as compared to the conventional methods. A similar study by [13] on the antimicrobial efficacy of *S. gabonensis* stem bark and the leaves of *Vernonia amygdalina* on two palm wine types *Elaeis guineensis* and *Raphia hookeri* was evaluated. Experimental results were expressed as mean and standard error of mean (SEM). Results were processed with MS-Excel 2007 and origin 6.0. Data were analyzed using analysis of variance (ANOVA). Findings revealed that *R. hookeri* sample has more heterotrophic bacteria and coliform counts than *E. guineensis* sample. *E, guineensis* sample has more yeast species than *R. hookeri* sample. Thus, addition of *S. gabonensis* and *V. amygdalina* lowered the growth of the isolated bacteria and fungi. There is reduction in CO_2 emission as well as pH stability.

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Phytochemical screening of *Sacoglottis gabonensis* bark extract reveals the presence of two major phenolic compounds. First is bergenin which is also known as Cuscutin with chemical formula $C_{14}H_{16}O_9.H_2O$, and IUPAC name as (2*R*,3*S*,4*S*,4a*R*,10b*S*)-3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,,4a,10b-tetrahydropyrano[3,2-*c*][2]benzopyran-6(2H)-one, and molar mass 346.3 g/mol, has been shown to exhibit antifungal, antitussive anti-inflammatory, anti-hepatotoxic, anti-alcerogenic, anti-HIV, immunodulatory, antiplasmodial, antiarrhythmic, antitumor antidiabetic activities as well as burn wound healing effect.

The second phenolic compound is garlic acid, a 3,4,5-trihydroxyl benzoic acid with chemical formula $C_6H_{12}(OH)_3COOH$, molar mass 170.12 g/mol is also found to be useful in pharmaceutical industries for the determination of phenol content of the various analytes by the Folin Ciocaltean essay as well as in some chemical synthesis.

2. MATERIALS AND METHODS

2.1 Definition of terms

TCC = Total coliform count, EMB = Eosine methylene blue, MA = MacConkey Agar

2.2 Samples Collection and Preparations

The materials used are the plant bark extract and the water samples. The bark of the plant was collected by a palm wine tapper and was confirmed by a botanist in Imo State Polytechnic, Omuma. The water samples were collected from Otamiri river and pond water in Mgbirichi, all in Imo State, southeastern Nigeria. The plant bark was washed with distilled and air dried for 5 days. A sterile mortar and pestle was used to mash the tree bark into pieces and was powdered using clean grinding machine. The two water samples were mixed together into a 1000 ml beaker. The extraction was done in three batches; cold, hot and alcoholic extracts. Each batch

consists of three beakers and all the beakers were labeled beakers 1-9, and the concentration of the plant extracts for cold and hot water extract were 0.1g, 0.2g, 0.3g respectively. To each of the six beakers, 10 ml of the water sample were added. The cold water extract were stirred for five minutes. The hot extracts were heated for five minutes using a bunsen burner. Then, the six beakers were made up to 100 ml with the water and contents were filtered into six other beakers. For the ethanol extract, 10 ml of the water sample were added to another three beakers (beakers 7-9) containing 0.1g, 0.2g, 0.3g of the tree back, the beakers were stirred for 10 minutes, made up to 100 ml with the sample, and then filtered into another three beakers. 10 ml of the water sample was added to 10ml of ethanol and was labeled 10. Into another sterile beaker, 10 ml of the water sample was added and was labeled 11.

2.3 Preparation of Media

About 15g of MacConkey agar (MA) and 12.2g of Eosine Methylene blue (EMB) respectively dissolved in 200ml of water were used to prepare the media. The conical flasks were swirled and foiled and autoclaved at 121^{o}Cfor 15 minutes.

2.4 Serial Dilution

The water samples (1, 2, 3...11) were serially diluted using distilled water. A 10 fold serial dilution according Uwuezuoke (2006) was adopted. 1ml of the water sample was pipetted into the first test tube containing 9ml of distilled water (stuck solution) and was labeled 10^{-1}. Into the second tube, I ml of the solution was aseptically drawn from the first tube containing 9ml of distilled water of the dilution series $10^{-2.}$ The dilution was continued till 10^{-10} .Thus, Dt= D1*D2*D3*…..Dn

Where Dt is the total dilution factor and Dn is the dilution ratio.

2.5 Determination of the Microbial Load and Isolation

The pour plate technique according to Uwuezuoke (2006) was adopted. A 1 ml factor was introduced in to two sterile petri dishes. 15ml of MacConkey agar and 15ml of Eoisine methylene blue were respectively added into the dishes. The media and the inoculum were mixed for 5 minutes. The sterile petri dishes were incubated for 3 hours at 37^{o}C and colonies were counted after 24hours.However, in order to determine the effect of the bitter back tree on the water sample, the total coliform count was determined at each concentration of the plant back in weight per volume and then compared to the count before treatment with the plant back.

CFU/ml = No of Colonies * Total dilution factor)/ Volume of culture plated in ml

Meanwhile, total coliform are standard by which microbial contamination is measured. Thus, coliform are the first microbes to be found in water hence, an indicator of possible contamination.

Table 1: Blank (contaminated water without treatment=control).

Sample	Beakers	Total coliform count
Water sample and ethanol	10	5
Water sample (blank)	11	45

Table 2: Cold Aqueous Extract

Concentration of plant extract w/v or g/ml	Sample/beakers	Total coliforms in Eosine methylene blue medium	Total coliform in macConkey medium
0.1	1	15	10
0.2	2	9	6
0.3	3	5	3

Table 3: Hot Aqueous Extract

Concentration of plant extract w/v or g/ml	Sample /Beakers	Total coliform count in Eosine Methylene blue	Total coliform count in macConkey medium
0.1	4	7	5
0.2	5	3	1
0.3	6	1	Nil

Table 4: Ethanol extract

Concentration of plant extract in w/v or g/ml	Sample / Beakers	Total coliform count in Eosine Methylene blue	Total coliform count in macConkey medium
0.1	7	3	Nil
0.2	8	Nil	Nil
0.3	9	Nil	Nil

3. RESULTS

Table 1 shows the total coliform count in the water without treatment with the plant bark extract. TCC in the untreated water (control) is 45 while TCC in the water sample mixed with ethanol is 5. Table 2 presents data of cold aqueous extract of the plant bark and TCC in EMB and MA media. At 0.1g, the TCC is 15 and 10 respectively. At 0.2g, TCC came down to 9 and 6 respectively, and at 0.3g, the count reduced to 5 and 3 respectively. Table 3 presents the data of hot aqueous extract of the plant bark and TCC in EMB and MA media. At 0.1g, TCC is 7 and 5, at 0.2g, it is 3 and 1, and then at 0.3g, it reduced to 1 and zero respectively. Table 4 shows data of alcoholic extract of the plant bark and TCC in both media. At 0.1g the count is 3 and 0 respectively. At 0.2g and 0.3g, there were no coliform found in both media.

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4. DISCUSION

Result showed that soaking any part of plant in suitable solvents such as water, ethanol etc activates the phytobiotic efficacy of such plant since their activity against microbes has been accredited to presence of phytochemical present in specific parts of plants .However, the effectiveness of the active ingredients on germs present in water is concentration and temperature dependent as well as solvent selective. The slight reduction of coliforms in cold extract is a clear indication of incomplete extraction, hence poor elimination. The moderate effect of hot extract in elimination of coliforms attests to the fact that most chemical reactions are temperature dependent. Hence, heating a plant extract induces the rate of reaction by breaking the intermolecular bonds hence greater felt effect. Meanwhile, total elimination of coliforms was observed in alcoholic extract compared to cold and hot aqueous extracts. A comparison of the effectiveness of the media indicates that there was more presence of coliforms in EMB than there is in MA. Analysis of table 4 shows that there was no growth of the coliforms in the MA media at all levels of operational concentrations. However, the choice of ethanol as supporting solvent is such that ethanol is life friendly, a good solvent for most substances and an efficient germ killer. The findings in the present study are in agreement with the claims of Maduka and Okoye [11] on antioxidant activity of S. gabonensis against lipid peroxidation, of Frank *et al.* [5] on antimicrobial activity against growth and proliferation of acetobacter species, Maduka and Okoye [10] on the protective effect of *S. gabonensis* and its isolate bergenin on RBC, PCV, HB against oxidants 2,4-DNPH and ethanol, and Madusolumuo and Okoye [9] on the effects of *S. gabonensis* stem bark extracts on anticoagulant properties and serum levels of acetylsalicyclic acid and acetaminophen. The findings in this work also align with that of [7] on reduced fermentation rate constant of palm wine by *S, gabonensis*. And that of [13] on antimicrobial efficacy of *S. gabonensis* and *V. amygdalina* on palm wine.

5. CONCLUSION AND RECOMMENDATION

Sacoglottis gabonensis bark extract treated water contains no coliform at 0.3 g as against 45 recorded in the control. The efficacy of *S. gabonensis* and other plant extracts on antimicrobial activities, antioxidant properties, anticoagulant properties, has been established by most studies. Hence, more exploration should be made on the inclusion of phytobiotic treatment as one of the safest and cheapest method of water purification in addition to other conventional methods. However, plant extract addition to potable water should fall between the recommendation ranges of WHO specification. Thus higher concentration of this extracts in water may affect the physicochemical qualities of water.

6. REFERENCES

Comment [a11]: Add recent references.

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