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. The study 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. 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/ml, 0.2 g/ml and 0.3 g/ml respectively using cold and hot water for aqueous extracts, while ethanol was used for alcoholic extract. 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 concentration. 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

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. Secondly, when these algae and water hyacinth die and decompose, this decay process uses a great amount of energy and causes death of aquatic organisms. However, phosphate contained in detergent contributes to the growth of algae in water bodies, resulting in overcrowding and death through insufficiency of sunlight. Bacteria action on these dead algae causes depletion of oxygen content of the water [1].

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, 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. A review of some related studies [2] indicates that plants possess multifaceted components which can be sustainably developed into product for water treatment. Results of the study on the antimicrobial activities of *Ocimum sanctum* leave extract on normal tap water and local river water showed that both samples recorded minimal microbial concentrations at 15 and 16 hrs of 500 mgl^{-1} and 600mgl^{-1} concentration of the plant extract [3].

Among evidences of plant phytoactivity against microbes include a report by Kirui *et al.* [4] on the efficacy of aqueous plants extract of *Acacia niloptica*, *Acacia segal*, *Acacia tortilis*, *Acacia etbiaca* and *Albiz anthelmintica* for water treatment capacity. The 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 [5]. 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. In a related study by Rama [6] herbal disinfection of water was carried out using *Oscimum Sanctum* using aqueous and alcoholic extract. In addition to the above literatures, Singh *et al.* [7] evaluated antimicrobial activities of 8 mixtures of different extract studied for their application to remove coliforms isolated in grey water and the use of thyme oil to treat E. coli in water.

Available data on cytoprotection *in vivo* and *in vitro* studies have shown the presence of bioactives in significant amount in both living things like human and rat, and in nonliving components such as palm wine and oils [8]. Some research scholars investigated the comparative effect of *S. gabonensis* stem back extract on reduced fermentation rate constant of palm wine [9]. Similar findings have also been reported by *Chijioke et al.* [10] on antimicrobial efficacy of *S. gabonensis* and *V. amygdalina* on palm wine.

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. The preservative effect of *S. gabonensis* back extract on biochemistry and sensory attributes of fermenting palm wine (raphia hookeri) has been previously reported [11]. Similarly, a report has been documented on the enhanced effect of the plant bark extract against membrane lipid peroxidation induced by 2, 4-dinitrophenyl hydrazine [12]. 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 Maduka and Okoye [13] 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. There is need to study this natural products in a view to 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.

Phytochemical screening of *Sacoglottis gabonensis* bark extract reveals the presence of two major phenolic compounds. First is bergenin which is also known as Cuscutin, ardisic acid B, bergenit, bergenitol, peltophorin, and vakerin 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. A review of the chemistry and pharmacology of bergenin or its derivatives as a promising molecule by Salimo *et al.* [14] identified bergenin as a triglycosides derivative of trihydroxybenzoic acid. It was first discovered in Russia in 1880 by Garreau and Machelart from rhizomes of the medicinal plant saxifrage crassifolia. It's believed that plant biosynthesize this molecule as an adaptive mechanism in response to abiotic and biotic stress. It has ability to attract animals and offers a UV protection. Another study revealed that bergenin has also been isolated in various parts of plants such as the root, Arial parts, tuber, flowers, rhizomes, stem, leaves, stem bark, heartwood, seed, leafy shoot, cortex, and in the whole plant. It is found in up to 112 plant species distributed in 34 plant families. It is a derivative of 4-O- methyl gallic acid, a hydrolysable phenolic glycoside obtained as a colorless crystal slightly soluble in water, degrades easily in basic solutions. It is stable at 80^o storage condition. Among other derivaties of bergenin with bioactivity include gallic acid, river bergenin, norbergenin, II-O-galloylbergenin, II-O-veratroylbergenin, 4-O- galloylnorbergenin, 8-O- methylnorbergenin, II-O- acetylbergenin, and II-O- vanilloylbergenin.

In previous studies, the stem back of *S gabonensis* was reported for its ability to reduce sperm concentration in rats, sperm immobilization potential, brain glucose protecting effect, natural antioxidant defense, and inhibition of white blood cell proliferation both in differential and total counts [15].

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 was adopted. A 1 ml factor was introduced in to two sterile petri dishes. 15ml of MacConkey agar and 15ml of Eosine 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: Variation of total coliform count with respect to water samples

Sample	**Beaker No.**	**Total coliform count**
Water sample and ethanol	10	5
Water sample (blank)	11	45

The data in Table 1 has been presented in Figure 1 below.

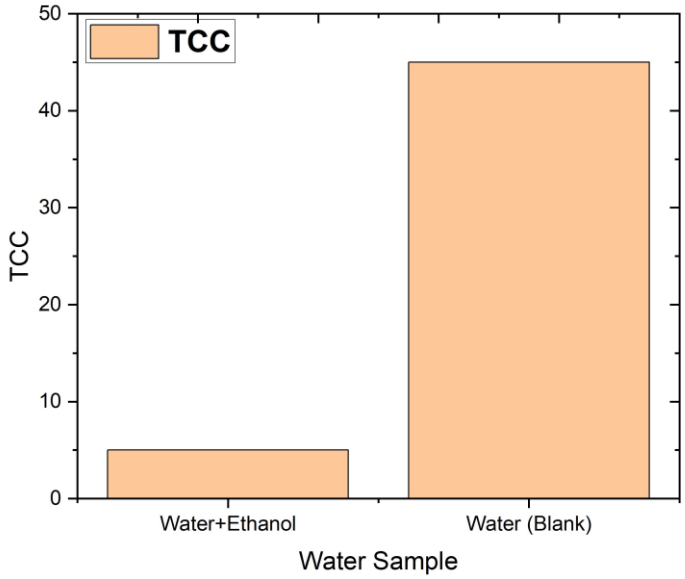

Figure 1. Variation of total coliform count (TCC) with respect to water samples

Table 2: Variation of total coliform count with extract concentration in the two media using cold aqueous extract

Concentration of plant extract (g/ml)	Sample/Beaker No.	Total coliform count in Eosine methylene Blue Medium	Total coliform count in MacConkey Medium
0.1	1	15	10
0.2	2	9	6
0.3	3	5	3

The data in Table 2 has been presented graphically in Figure 2 below.

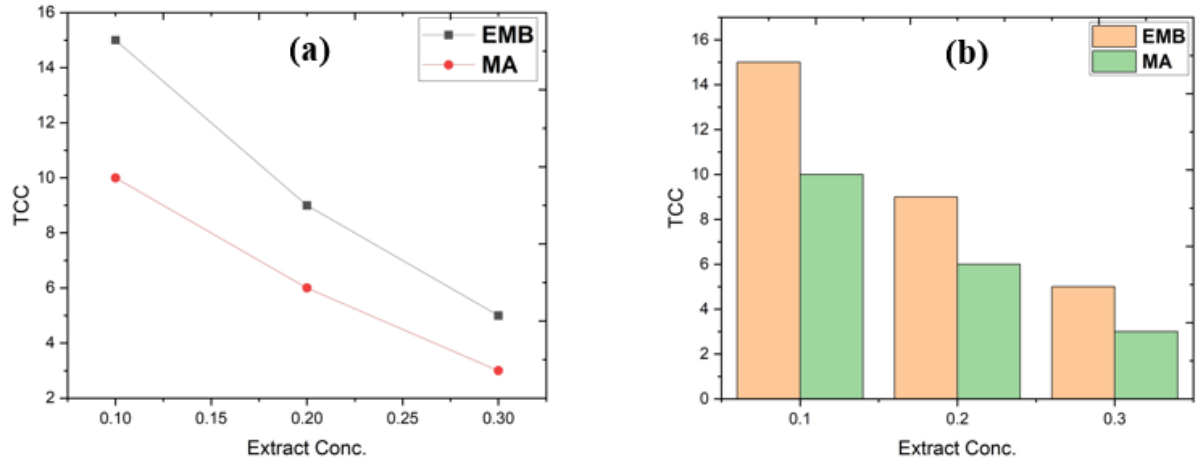

Figure 2. Variation of total coliform count with media (a) Graph (b) Bar chart

Table 3: Variation of total coliform count with extract concentration in the two media using hot aqueous extract

Concentration of plant extract (g/ml)	Sample /Beaker No.	Total coliform count in Eosine Methylene Blue Medium	Total coliform count in MacConkey Medium
0.1	4	7	5
0.2	5	3	1
0.3	6	1	Nil

The data in Table 3 has been presented graphically in Figure 3 below.

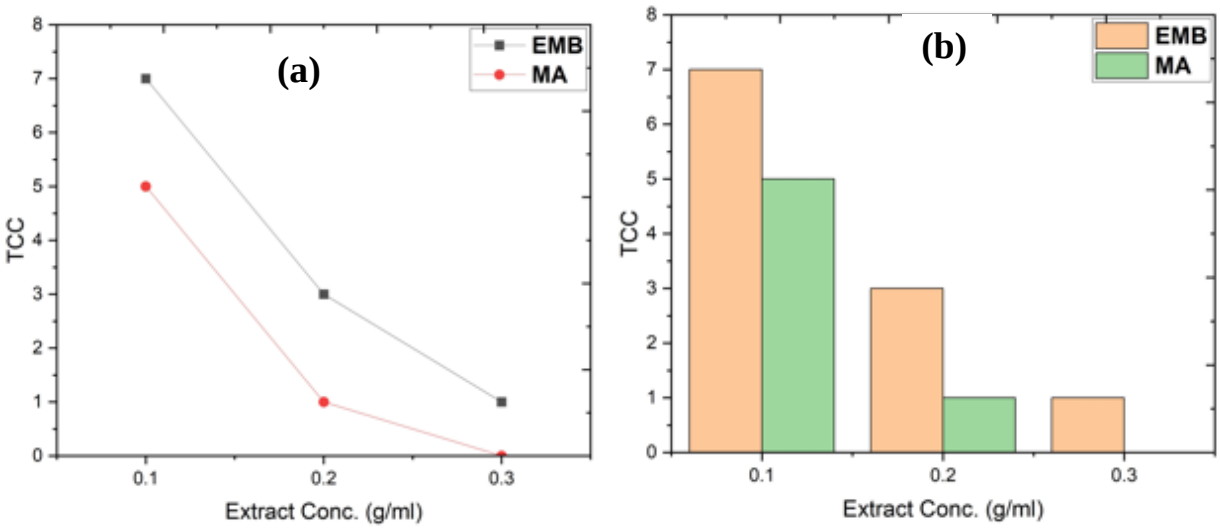

Figure 3. Variation of total coliform count with media using hot aqeuous extract (a) Graph (b) Bar chart

Table 4: Variation of total coliform count with extract concentration in the two media using ethanol extract

Concentration of plant extract (g/ml)	Sample /Beaker No.	Total coliform count in Eosine Methylene Blue Medium	Total coliform count in MacConkey Medium
0.1	7	3	Nil
0.2	8	Nil	Nil
0.3	9	Nil	Nil

The data in Table 4 has been presented graphically in Figure 4 below.

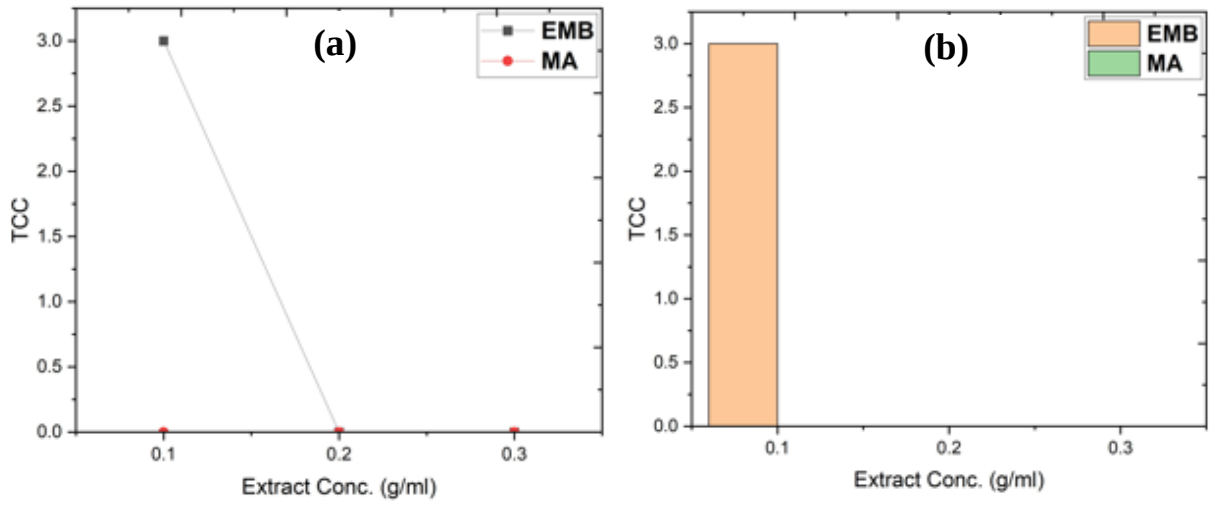

Figure 4. Variation of total coliform count with media using ethanol extract (a) Graph (b) Bar chart

3. RESULTS AND DISCUSSION

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 in EMB and MA respectively. At 0.2g, TCC came down to 9 and 6 in EMB and MA respectively, and at 0.3g, the count reduced further to 5 and 3 in EMB and MA 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.

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 the presence of phytochemicals 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 influences the rate of reaction by breaking the intermolecular bonds hence greater 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 by Adeyemi *et al.* [2] revealed that seed (24.58%), flower (20.75%), leaf (16.98%), resin (1.89%), bark (1.89%) and tuber (1.89%) are useful tools 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%). The findings in the present study conforms with that of Kirui *et al.* [4] on the efficacy of aqueous plants extract of *Acacia niloptica*, *Acacia segal*, *Acacia tortilis*, *Acacia etbiaca* and *Albiz anthelmintica* for water treatment capacity. The report indicates a notable effect towards inhibiting the proliferation and eradication of microbes. Similrly, *Moringa oleifera*, *Jatropha curcas* and quar gum was investigated by Abantneh *et al.* [5] for their water treatment abilities; it was observed that there is a reduction in turbidity of the treated water as against the untreated control. Some researchers also recorded higher inactivation of the E.coli when compared to Ozonation and Chlorination by the utilization of 8 mixtures of different plant extracts studied for their application in water treatment [7].

Results of a related study by Rama *et al.* [6] following a similar methodology to disinfect water using aqueous and alcoholic extract of *50g of Ocimum Sanctum* dry leaf 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. The antimicrobial actions of Tulsi (*Oscimum Sanctum)* leaf extract with different concentration (100-600mgl^{-1}) in tap and river water has been previously reported [3]. It was found out that 600mgl^{-1} conc. of plant extract treated water showed effective antimicrobial activity at 15 and 16hrs than the other concentration of extract. The 500mgl^{-1} treated water showed 95%-98% antimicrobial activity in 14 and 16hrs. 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 was also investigated elsewhere [9]. The rate constant for *S. gabonensis*, *A. boonei* and the control were $1.1x10^{-4}$ mol $^{-1}$ s^{-1}, 1.7×10^{-4} $m^{-1}s^{-1}$ and 2.79×10^{-4} $mol^{-1}s^{-1}$ respectively. However, yeast growth was enhanced in the ratio of 0.43: 0.76: 0.88 respectively.

Thus, the findings by Philippa [11] on antimicrobial efficacy of *S. gabonensis* bark extract conforms to the findings of present study. In the study, alcoholic content, refractive index, assimilation of sugars, and nitrates were monitored (for 4 days) in both the treated sample and untreated control. Fermentation rate were monitored by determining the CO_2 production. The fermenting samples and acidity changes were also monitored within the same period. The pH of the samples was determined on daily basis using pH meter. The sensory parameters (taste, color aroma) were monitored for possible changes. Result showed that alcohol content of both

preserved and unpreserved samples were increased up to the fourth day after which alcohol content decreased. However, the decreased alcohol content signifies conversion of alcohol to vinegar. Meanwhile, the alcohol content of the sample containing > 490-510mgl-^{1} was generally higher than that of unpreserved sample. Secondly, there was significant difference ($p \leq 0.05$) in the refractive index. Sugar qualities decreased more in samples containing *S. gabonensis* than in unpreserved samples. This implies that *S. gabonensis* bark extract lower sugar content of alcohols and reduces CO_2 production. In addition to the concurring literatures, A similar study on the cytotoxic activity of *S. gabonensis* stem bark extract against mouse lymphoma cell line L. has been reported [15], In the study, three secondary metabolites namely, methyl 3, 4-dihydroxy-4- methoxybenzoate, Eriodictyol and bergemin were isolated, and characterized using normal hexane, EtOAc (ethyl acetate) and normal butanol respectively. HPLC and TLC were employed during extraction, isolation while NMR was used to determine the molecular weights of the isolates. The cytotoxicity essay was carried out. Result showed that compound 1 is a yellow solid characterized to be methyl 3, 4-dihydroxy-4- methoxybenzoate has absorption peak 260.5 and 298nm with molecular structure $C_9H_{10}O_5$ and molar mass 198gmol-^{1} confirmed by MS spectra data. Compound 2, a light yellowish white solid characterized as eriodictol. Its UV maximum absorbance is 288nm with molar formula $C_{15}H_{12}O_6$ provided by ESI-MS spectra and molar mass 288gmol-^{1}. The third compound, a white crystalline solid characterized to be bergenin with UV max peaks of 230,328nm with molecular formula $C_{14}H_{16}O_9$ and molar mass 328gmol-^{1}. However, eriodictol and bergenin were weakly active and showed a growth inhibition of 27.3 and 15.2% when tested for their cytotoxic activity against the mouse lymphoma cell line L

In addition, result of the this study is in line with that of Frank and Idorienyin [8] on the effect on *S. gabonensis* bark extract on shelf life of palm wine, 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. A similar study on the antimicrobial efficacy of *S. gabonensis* stem bark and the leaves of *Vernonia amygdalina* on two palm wine types *Elaeis guineensis* and *RapBabihia hookeri* was evaluated by [10]. The 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. The findings in the present study are in agreement with the claims of [12] on antioxidant activity of *S. gabonensis* against lipid peroxidation. A study on the protective effect of *S. gabonensis* and its isolate bergenin on RBC, PCV, HB against oxidants 2, 4-DNPH and ethanol was also been reported [13].

5. CONCLUSION

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. I *S gabonensis* bark extract treated water has no side effect and the have received increasing attention due to presence of bergenin and its derivatives in food and medicinal plants. Generally speaking, *S.gabonensis* possesses antifungal, antitussive, anti-inflammatory, anti-hepatotoxic, anti-alcerogenic, anti-HIV, immunodulatory, antiplasmodial, antiarrhythmic, antitumor antidiabetic activities as well as burn wound healing effect. These features are attributed to the presence of bergenin with multi-functional ability both in living and in non-living components as well as in vivo and in vitro. Its efficacy in prolonging the shelf life of vegetable oils, alcoholic, and nonalcoholic beverages has been established. It is cytoprotective and offers numerous antioxidant opportunities. In addition, *S. gabonensis* water treatment is simple, cost effective easily assessable and environmental friendly. It reduces rate of CO_2 emission, reduces sugar content of palm wines and stabilizes pH of its solutions. *S gabonensis* treated water serve not only as germ killer but also as medicinal water as opined by [3].

6. RECOMMENDATION

This study recommends that more exploration should be made on the inclusion of phytobiotic treatment with reference to bergenin 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.

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