



## JOURNAL OF TECHNICAL REPORTS IN ENGINEERING AND APPLIED SCIENCE

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### Investigation of some selected plant extracts for the control of halitosis

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#### ARTICLE INFO

#### ABSTRACT

#### ORIGINAL RESEARCH ARTICLE

##### Article History

Received: April 2016

Accepted: April 2016

##### Keywords:

Halitosis,  
Bacteria,  
microorganism,  
extracts.

Halitosis or bad breath is an unpleasant or offensive odour of the mouth emanating from oral cavity present on exhaled breath. According to medical experts, this very embarrassing condition which can be acute or chronic could be caused by several factors including food, poor dental hygiene, tobacco products, health problems, dry mouth, infections, and medications. Halitosis is detrimental to one's self-image and confidence causing social, emotional and psychological anxiety. Some food products (barley tea, green tea etc) and plant extracts (Eucalyptus, ginger, aloe vera and coconut) are claimed to be effective in controlling halitosis. To this notice, investigation of some selected plant extracts is conceived as an efficient way to control halitosis. This study was carried out to investigate the mixed extract of *Eucalyptus globulus*, ginger, aloe vera and coconut oil for the control of halitosis. Organolyptic measurements were used to check for the presence of halitosis. There were 24 volunteers from Science laboratory Technology Federal Polytechnic Idah, aged between 20 to 25 years old. The result of the first examiner before administration of the mouth wash showed that 3(12.5%) absence, moderate and strong malodour, 2(8.30%) had questionable malodour, 6(25.00%) had slight malodour, and 7(29.16%) had severe malodour whereas the result after the administration of the mouth wash showed that 14(58.33%) had absence of malodour, 4(16.67) had questionable and slight malodour, 0(0.00%) had moderate and strong malodour and 2(8.30%) had strong malodour. The result of the second examiner before the administration of the mouth wash showed that 3(12.50%) had absence, questionable, moderate and strong malodour, 5(20.83%) had slight malodour and 7(29.16%) had severe malodour whereas the result after the administration of the mouth wash showed that 14(58.33%) had absence of malodour, 2(8.30%) had questionable malodour, 5(20.83%) had slight malodour, 0(0.00%) had moderate, strong and severe malodour. The antimicrobial activity of the mixed extract against

*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was determined and their zones of inhibitions were 16, 17, 14 and 13mm respectively at 80ul. The mixed extract was active against the microorganisms which are causative agent of halitosis in vitro. There was great reduction in the number of volunteers who had halitosis when the mixed extract was administered. So the mixed extract was effective against the microorganisms which cause halitosis in vivo. It was also able to give fresh breath and reduce oral malodour

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## 1. Introduction

Humans emit a variety of volatile and non volatile molecules that are influenced by genetics, diet, stress and disease<sup>1</sup>. Halitosis is an unpleasant or offensive odour emanating from oral cavity. Halitosis, factor oris, oral malodour or bad breath are the general terms used to describe unpleasant breath emitted from a person's mouth regardless of whether the odorous substance in the breath originate from oral or non oral sources. Halitosis or bad breath is an oral health condition characterized by unpleasant odours emanating consistently from the oral cavity. The origin of halitosis may be related both to systemic and oral conditions, but a large percentage of cases, about 85%, are generally related to oral cause<sup>2</sup>.

Causes of halitosis include; poor oral health care, closure of mouth for a very long time, improper cleaning of the dentures, certain foods like garlic, decreased salivary flow rate, tobacco products or medical conditions, microbial degradation of organic substrate present in saliva and debris, bacteria coating of tongue. The tongue is the major site of oral malodour production<sup>2</sup>.

The principle component of halitosis is volatile sulphide compounds, especially hydrogen sulphide, (H<sub>2</sub>S), methyl mercaptan (CH<sub>3</sub>SH) and dimethyl sulphide (CH<sub>3</sub>SCH<sub>3</sub>). Organolyptic measurement, sulphide monitoring and gas chromatography are used for the measurement of halitosis<sup>3</sup>. At least 50% of the population suffers from chronic oral malodour and approximately

half of these individuals experience a severe problem that creates personal discomfort and social embarrassment. Chemical rinses like Chlorohexidine mouthwash, Ceptylpyridinium mouthwash have been used for the control of halitosis<sup>4</sup>.

These chemical rinses are somewhat expensive and have some negative effect on human. Some of them are not common in some remote areas, so there is need to look into some plants that have antimicrobial activity and that are capable of giving fresh breath like *Eucalyptus globulus*, Aloe vera, Ginger and coconut.

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs<sup>5</sup>. Plants are considered as largely complicated chemical factories which can turn the simple ingredients of air and water into many compounds including liquids and water into many compounds including liquid and oils. Plants have been serving the animal kingdom as its source of energy (food, fuel) as well as its means of shelter. In addition to the source of energy, plants have been synthesizing a large variety of chemical substances. These substances in addition to basic metabolites include phenolic compounds terpenes, steroid, alkaloids and other chemical substances which are known as secondary metabolites which have prominent effect on the animal system and

some possesses important therapeutic properties which can be and have been utilized in the treatment and cure of human and other animals' diseases for many centuries. Secondary metabolites differ from plants to plants. The plants which produce and accumulate constituents have medicinal values and are generally designated as medicinal plants<sup>6</sup>.

*Eucalyptus globulus* is an important plant belonging to the family *myrtaceae*. The name originates from the Greek word "eucalyptol" which means well "covered". It is a large genus of aromatic trees indigenous to Australia, Tasmania and the neighboring Island, but today, it can be found growing in subtropical regions of the world. The genus consists of about 700 species of evergreen trees and shrubs<sup>5</sup>.

However, the prevalence of microbial resistances to existing microbial drugs underscores the need for the continuous search for new antimicrobials. One of the alternatives for such search is to screen medicinal plants for antimicrobial activities<sup>7</sup>. *Eucalyptus* fresh leaves have eucalyptol (1,8 sineole) as the active ingredient and this is responsible for its various pharmacological actions<sup>5</sup>. *Aloe vera* Linne or *Aloe barbadensis miller* is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The innermost part of the leaf is a clear, soft, moist and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of viscous mucilage. Therefore, the thick fleshy leaves of *aloe vera* plants contain not only cell wall carbohydrates such as cellulose and hemicellulose but also storage carbohydrates such as acetylated mannans<sup>8</sup>. As a drink it protects the mucous membrane of the stomach especially when irritated or damaged. It has antibacterial properties. *Aloe vera* juice is considered helpful for

relieving many types of gastro intestinal irritation and juice products are widely available<sup>9</sup>.

*Cocos nucifera* (coconut) belongs to the family *Aracaceae*. The plant is mainly used as a staple food crop and a source of wood and handicrafts, among many other uses, and is thought by many to be the world's most useful plant and medicinal plant in tropical and subtropical countries. *Cocos nucifera* is found throughout the tropics, where it is interwoven into the lives of the local people<sup>10</sup>.

According to<sup>11</sup> in his studies, the traditional medicine in North-Eastern Brazil, coconut husks have been used for the treatment of diarrhea and arthritis.

Nowadays, coconut oil obtained from the fruit of coconut palm, has been relegated mainly to non food uses in the developed countries but retains its importance for traditional uses in producing countries. Coconut oil has been confirmed to possess antimicrobial, antiviral and antiprotozoal activities<sup>12</sup>.

Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation and infectious diseases. Ginger has direct antimicrobial activity and thus can be used in treatment of bacterial infections. Ginger belongs to the family *Zingiberaceae*. The zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non tuberous rhizomes<sup>13</sup>.

Medicinal plants contain large varieties of chemical substances with important therapeutic properties that can be utilized in the treatment of human diseases. The prevalence of halitosis has been reported to be as high as 50% of a population<sup>14</sup>. Halitosis is detrimental to one's image and

confidence causing social, emotional and psychological anxiety. It causes social embarrassment and personal discomfort and has caused otherwise well qualified people lose job opportunities, friendships and even intending couples. Consequently, there is the increasing justified assumption which claims that traditional medicine is cheaper and more effective than modern medicine. The studies of medicinal plants used as folklore remedies have therefore attracted immense attention in the scientific world in an attempt to find possible solutions to the problems of multiple resistances to the existing synthetic and conventional antimicrobials<sup>10</sup>. The increase usage of antibiotics has induced microorganism to acquire resistance factors which have become a burning predicament. As a result there is an urgent need to find the alternative of chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have considerably less side effects and are capable of given fresh breath and are of antimicrobial properties against organism associated with halitosis.

The aim of this study is to produce mouthwash from eucalyptus leave, aloe vera, ginger and coconut oil for the control of halitosis.

The following are the objectives of this study;

- To extract oil from coconut and produce extract from *Eucalyptus globulus* leave, Ginger and Aloe vera.
- To carry out antimicrobial activities of the mouthwash against selected organisms (Escherichia coli, Staphylocococcus aureus, and Pseudomonas aeruginosa and klebsiella pneumoniae).
- To inhibit the growth of micro organism causing halitosis.
- To suppress volatile sulphide compound in the mouth.

## 2. Methodology

The materials used for this project work include Soxhlet extractor, Mortar and pestle, Eucalyptus leaves, Coconut, Aloe vera, Ginger, water, Ethanol, baking soda, Mueller Hinton Agar, Petri dishes, Cotton wool, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

### Collection of Plants Materials

Eucalyptus leaves were collected in Bishop's house, Idah town Kogi State. Coconut was bought from Ega market in Kogi State while ginger and aloe vera were collected from Staff quarter Federal polytechnic Idah Kogi State. The Eucalyptus leaf was identified by a botanist in the University of Nigeria, Nsukka.

### Test Organism

Typed culture of bacterial and fungal isolates *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were collected from Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Federal University of Ibadan.

### Preparation Of Eucalyptus Extract

The eucalyptus leaves were washed with water dried for seven (7) days. They were then crushed using mortar and pestle. 50g of the crushed sample was subjected to soxhlet extraction using 400ml of water. This was done by boiling it with Soxhlet apparatus on its working on steam distillation process for 10 hours. The working temperature was maintained at 100 °C and was distilled for one (1) hour<sup>15</sup>.

### Extraction of Coconut Oil

The coconut water was collected by crack opening of the shell. Coconut meat was extracted from the shell using a clean sharp knife. A vegetable peeler was used to peel off the brown skin. The coconut was rinsed with water and ground using mortar and pestle. This was mixed thoroughly with the coconut water and one (1) litre of clean

water, using bare hand to squeeze it very well, until the mixture looked very white and creamy. The mixture was sieved using white cheese cloth. The filtrate was allowed to stand in a bowl for 48 hours and then put in the fridge for 3 hours. This led to the formation of a layer of solid white mass at the top which was the oil. This was carefully extracted from the remaining liquid using a big rubber spoon. The oil was then heated for 20 minutes and was stored in a clean white rubber for further use<sup>16</sup>.

#### **Preparation of Ginger Extract**

Fresh ginger (*Zingiber officinale*) was peeled and washed with water. It was wet-milled using electric blender. The smooth paste without addition of water was sieved using cheese cloth. The raw extract was stored in plastic jars in the refrigerator for further use<sup>17</sup>.

#### **Preparation of Aloe Vera gel**

Mature and fresh leaves of aloe vera were washed in the running tap water for 5 minutes and rinsed with distilled water. Then it was dissolved longitudinally and the colourless parenchymatous tissue (aloe vera gel) was scraped out using a sterile knife without the fiber. It was ground with mortar and pestle and then sieved with cheesecloth<sup>18</sup>.

#### **Production of the Mouthwash**

The whole extract (ginger paste, aloe vera gel, eucalyptus extract and coconut oil) were mixed in different proportions<sup>19</sup>.

Composition of the mouthwash:

Each 1000ml of the mouthwash contains

Ginger paste	20ml
Aloe vera gel	7ml
Eucalyptus extracts	15.5g
Coconut oil	15ml
Ethanol	1.5ml
Baking soda	1g
Distilled water	957ml

#### **Determination of antimicrobial activities and minimum inhibitory concentration test by agar diffusion technique.**

Antimicrobial activity of the extract (mouthwash) was determined by the method described by<sup>20</sup>. Requisite quantity of Mueller Hinton Agar media (Biotech India) was poured in the sterile Petri dish. After solidification, test organism was spread over the solidified Mueller Hinton agar and the Petri dishes were incubated at 37°C for 24 hours to grow the microorganism. With the help of sterile Cork borer (6mm) a hole was made on the media and known concentration 30-80ul test solution was poured in the hole. The same presence was repeated for standard drug. The diameter of zone of inhibition of both test solution and standard antibiotics were measure at 24 hours. The lowest concentration of the extract mixture that inhibited the test organism was taken as the Minimum Inhibitory Concentration (MIC) for each test organism.

#### **Test for Halitosis**

Measurement was conducted early in the morning around 6:30am. 24 students of Science Laboratory Technology of Federal Polytechnic Idah aged 20-25 years were selected and grouped into four, ( six (6) in a group). Organoliptic measurement was used in conducting the test. The students were asked to abstain from the following; taking antibiotics two weeks before the test, eating garlic, onions and spicy foods for 48 hours before the assessment and using scented cosmetics for 24 hours before the assessment. They were further instructed to abstain from the following for 12 hours before the assessment; eating any food or drink, using their usual oral hygiene practices, using oral rinses and breath fresheners, and smoking. The examiners also refrained from drinking tea, coffee or juice, smoking and using scented cosmetics before the assessment.

Two organoliptic measurement methods were used simultaneously by two examiners. Normal breath was examined by smelling while the relaxed seated subject was



exhaling slowly and powerfully. For the spoon test, the tongue dorsum was scraped with cotton wool and the scraped material was smelled after 20 seconds. Organoleptic measurements were recorded as 0(no malodour or absence of odour),

1(questionable malodour), 2(slight malodour), 3(moderate malodour), 4(strong malodour), 5(severe malodour)<sup>21</sup>. One examiner did normal breath and the second did spoon test.

#### Administration Of The Mouthwash

Group	Morning	Afternoon	Night
A	X	X	X
B	Y	Y	Y
C	Y	X	Y
D	X	X	Y

X = not using the mouthwash.

Y = using the mouthwash.

Group A did not use the mouthwash so they served as the control group. Group B used the mouthwash three times per day, Group C used the mouthwash twice per day and Group D used the mouthwash just once per day. This lasted for seven days and the test of the mouth wash and Tables 3 and 4 shows the result obtained by the examiners after administration of the mouth wash. Also, the results of antibacterial investigations are

above was repeated by the two examiners and result was recorded.

#### 3 Results and Discussion

Table 1 and 2 shows the results of test obtained by the two examiners before the administration given in table 5 which indicates that different bacterial species exhibited different levels of sensitivities towards the different concentration of the mouth wash.

**Table 1:** Prevalence, percentage, mean scores and standard deviations of normal breath before administration of mouth wash by first examiner

Group	Scale	Category	Normal breath
Group A	0	Absence of malodour	1(17%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	3(50%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	2.8(1.8)
Group B	0	Absence of malodour	0(0%)
	1	Questionable malodour	1(17%)
	2	Slight malodour	2(33%)
	3	Moderate malodour	1(17%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	3.0(1.53)
	0	Absence of malodour	0(0%)

Group C	1	Questionable malodour	0(0%)
	2	Slight malodour	0(0%)
	3	Moderate malodour	2(33%)
	4	Strong malodour	1(17%)
	5	Severe malodour	3(50%)
		Total	6(100%)
		Mean (s.d)	4.2(0.9)
Group D	0	Absence of malodour	2(33%)
	1	Questionable malodour	1(17%)
	2	Slight malodour	1(17%)
	3	Strong malodour	0(0%)
	4	Strong malodour	2(33%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	1.8(1.69)

**Table2:** Prevalence, percentage, mean scores and standard deviations of spoon test before administration of mouth wash by second examiner

Group	Scale	Category	Spoon test
Group A	0	Absence of malodour	1(17%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	2(33%)
	3	Moderate malodour	1(17%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	2.8(1.8)
Group B	0	Absence of malodour	0(0%)
	1	Questionable malodour	1(17%)
	2	Slight malodour	2(33%)
	3	Moderate malodour	1(17%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	3.0(1.5)
Group C	0	Absence of malodour	0(0%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	1(17%)
	3	Moderate malodour	1(17%)
	4	Strong malodour	1(17%)
	5	Severe malodour	3(50%)
		Total	6(100%)

		Mean (s.d)	4.0(1.2)
Group D	0	Absence of malodour	2(33%)
	1	Questionable malodour	2(33%)
	2	Slight malodour	0(0%)
	3	Strong malodour	0(0%)
	4	Strong malodour	2(33%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	1.7(1.7)

**Table 3:** Prevalence, percentage, mean scores and standard deviations of normal breath after administration of mouth wash by first examiner

Group	Scale	Category	Normal breath
Group A	0	Absence of malodour	1(17%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	3(50%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	2.8(1.8)
Group B	0	Absence of malodour	6(100%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	0(0%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0(0)
Group C	0	Absence of malodour	4(67%)
	1	Questionable malodour	2(33%)
	2	Slight malodour	0(0%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0.3(0.9)
Group D	0	Absence of malodour	3(50%)
	1	Questionable malodour	2(33%)
	2	Slight malodour	1(17%)
	3	Strong malodour	0(0%)



	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0.7(0.9)

**Table 4:** Prevalence, percentage, mean scores and standard deviations of spoon test after administration of mouth wash by second examiner

Group	Scale	Category	Spoon test
Group A	0	Absence of malodour	1(17%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	2(33%)
	3	Moderate malodour	1(17%)
	4	Strong malodour	0(0%)
	5	Severe malodour	2(33%)
		Total	6(100%)
		Mean (s.d)	2.8(1.8)
Group B	0	Absence of malodour	5(83%)
	1	Questionable malodour	1(17%)
	2	Slight malodour	0(0%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0.2(0.4)
Group C	0	Absence of malodour	4(66%)
	1	Questionable malodour	1(17%)
	2	Slight malodour	1(17%)
	3	Moderate malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0.5(0.7)
Group D	0	Absence of malodour	4(67%)
	1	Questionable malodour	0(0%)
	2	Slight malodour	2(33%)
	3	Strong malodour	0(0%)
	4	Strong malodour	0(0%)
	5	Severe malodour	0(0%)
		Total	6(100%)
		Mean (s.d)	0.7(0.9)

**Table 5:** Antimicrobial activities of the mixed extract (mouth wash).

Sample	Concentration (ul)	Zone of inhibition (mm)			
		E.C	S.A	P.A	K.P
Mouthwash	30	11	12	10	9
	40	12	13	10	10
	50	12	14	12	10
	60	13	14	13	12
	70	14	16	13	13
	80	16	17	14	13
+ve control tetracycline	250mg/10ml	24	29	22	20
-ve control distilled water	Nil	Nil	Nil	Nil	Nil

E.C = *Escherichia coli*S.A = *Staphylococcus aureus*P.A = *Pseudomonas aeruginosa*K.P = *Klebsiella pneumoniae*

Nil = No inhibition

**Discussion**

Testing of the bad breath and the administration of the mouth wash was performed by two examiners for accuracy purposes. Tables 1 and 2 show the result of test obtained by the two examiners. Table 1 was obtained by examiner 1 while Table 2 was obtained by examiner 2. Each table was made up of four (4) groups (group A, B, C and D). Group A is used as the control experiment because they did not receive any treatment. Group B received treatment three times per day. Group C received treatment twice. Group D received treatment once. Each group was made up of six (6) volunteers. The two examiners used different methods, 5-point scale in the recording of the result of the test obtained. Examiner 1 used the normal breath test method while the examiner 2 used the spoon test method to test for halitosis. From the result obtained by the first examiner, group A; 1(17%) absence of malodour, 3(50%) slight malodour, 2(33%) severe malodour and nobody 0(0%) questionable and

moderate and strong malodour. The mean score and standard deviation was 2.8(1.8). Group B, 1(17%) questionable malodour, 2(33%) slight malodour, 1(17%) moderate malodour, 2(33%) severe malodour, and nobody 0(0%) absence and strong malodour.

Group C, 2(33%) moderate malodour, 1(17%) strong malodour, 3(50%) severe malodour while nobody 0(0%) absence, questionable and slight malodour. Group D, 2(33%) absence malodour, 1(17%) questionable malodour, 1(17%) slight malodour, 2(33%) strong malodour, and nobody 0(0%) has moderate and severe malodour.

The second examiner used spoon test for the halitosis. The following result were recorded, Group A 1(17%) absence and moderate malodour, 2(33%) slight malodour, 2(33%) severe malodour and nobody 0(0%) questionable and strong malodour. Group B, 1(17%) questionable malodour, 2(33%) slight malodour, 1(17%) moderate malodour, 2(33%) severe

malodour, and nobody 0(0%) absence and strong malodour.

Group C, 1(17%) slight, moderate malodour and strong malodour, 3(50%) severe malodour while nobody 0(0%) absence, questionable malodour. Group D, 2(33%) absence malodour, questionable malodour and strong malodour while nobody 0(0%) has slight malodour, moderate and severe malodour.

Notice: there is little/slight change but not noticeable in the result of the test conducted by the two examiners using the different method; normal breath and spoon test. Even in the mean score and standard deviation, the change is very small (+\_0.2). This shows the accuracy and correlation of the test and that organoliptic method is still the golden-standard.

Table 3 and 4 show the result of test obtained by the two examiners after the administration of the mouth wash. Table 3 was obtained by examiner one while table 4 was obtained by examiner two. It is noticed from table 3 that the group A (control experiment) of both examiner, has the same result as before the administration of the mouth wash. This is because the people in group A did not receive any treatment. While for other groups (B, C and D) of both examiners, there was a great reduction in the number of people suffering different categories of halitosis depending on the number of times people in the particular group received treatment.

From examiner one, (table 3); group B has 6(100%) absence of malodour while nobody 0(0%) has questionable, slight, moderate, strong and severe malodour. Group C; 4(67%) absence of malodour, 2(33%) questionable malodour, while nobody 0(0%) has slight, moderate, strong and severe malodour. Group D has 3(50%) absence of malodour, 2(33%) questionable malodour, 1(17%) slight malodour and nobody 0(0%) has moderate, strong and severe malodour.

Looking these result (group B, C and D), the mouth wash has the greatest effect on group B followed by group C and then group D. this is because group B received the highest number of treatment(three times) followed by group C(twice) and then group D(once).

For examiner two (table 4), group B has 5(83%) absence of malodour, 1(17%) questionable malodour while nobody 0(0%) has slight, moderate, strong and severe malodour. Group C; 4(66%) absence of malodour, 1(17%) questionable malodour, 1(17%) slight malodour while nobody 0(0%) has moderate, strong and severe malodour. Group D has 4(67%) absence of malodour, 2(33%) slight malodour and nobody 0(0%) has moderate, questionable, strong and severe malodour. Looking these result (group B, C and D), the mouth wash has the greatest effect on group B followed by group C and then group D. This is because group B received the highest number of treatment (three times) followed by group C (twice) and then group D (once). In general, there is correlation in the result obtained by the two examiners.

Also, the antimicrobial activity of the mixed extract was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The result of the sensitivity test indicates that different bacterial species exhibited different levels of sensitivity towards the different concentration of the mouth wash.

The different concentration of mouth wash has different sensitivity towards gram positive and gram negative bacteria. The sensitivity towards the microorganism was increased as concentration increases. The table 5 shows that the mouth wash has (30-80ul) maximum sensitivity toward *staphylococcus aureus*, while it has low sensitivity towards *Klebsiella pneumonia*. However, the mouthwash was effective in inhibiting the four test organism with zone of inhibition ranging between 9mm and

29mm. This shows that the results obtained from the antimicrobial activity of the mixed extracts of Eucalyptus leave, Aloe vera, Ginger and Coconut oil which can be attributed to the action of the phytochemical compounds it contains<sup>22</sup>. The sensitivity test also proves that the mouth wash was able to reduce halitosis. This is because; the microorganisms which cause halitosis were inhibited by the mixed extracts in vitro. The mouthwash was also able to reduce oral malodour, freshen breath and inhibit the microorganism that causes halitosis in vivo.

#### 4. Conclusion

From the analysis and results above, we have been able to investigate how the selected extract (Eucalyptus leave, Aloe vera, Ginger and Coconut oil) can be used to make mouthwash that could be used to control halitosis. The work also showed how microorganisms like Enterobacteroseae (*Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*) which cause halitosis were inhibited by the mixed extracts in vitro and how they were inhibited in vivo. Therefore, with the help of antimicrobial activity, we have seen that the mixed extract of Eucalyptus leaf, Aloe vera, Ginger and Coconut oil has great potential as antimicrobial agents in the control of halitosis.

#### Acknowledgements

The Technical support of the technologists at Chemistry and Microbiology laboratory, Department of Science Laboratory Technology, Federal Polytechnic Idah is acknowledged.

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