EVALUATION OF SOME SELECTED PLANT EXTRACTS AGAINST HALITOSIS CAUSING ORGANISMS

Fatai Abiola Lateef, Blessing Uloma Eze*, Zacchariah Egbunu, Paul Jere and Danlandi Yusuf
Department of Science Laboratory Technology,
Federal Polytechnic P.M.B.1037, Idah, Kogi State, Nigeria.
e-mail: visituloma@gmail.com

ABSTRACT

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 aeurginosa and Klebsiella pneumonia* was determined and their zones of inhibitions were 16, 17, 14 and 13 mm respectively at 80ul. The mixed extract was active against the microorganism 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

Keywords: Halitosis, bacteria, microorganism, extracts, malodour

INTRODUCTION

Humans emit a variety of volatile and non volatile molecules that are influenced by genetics, diet, stress and disease (Crispian and Greenman, 2008). Halitosis is an unpleasant or

offensive odour emanating from oral cavity. Halitosis, fector 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 (Jose *et al.,* 2008).

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 (Jose *et al.,* 2008). The principle component of halitosis is volatile sulphide compounds, especially hydrogen sulphide, (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulphide (CH_3SCH_3). Organolyptic measurement, sulphide monitoring and gas chromatography are used for the measurement of halitosis (Adewole *et al.,* 2014). 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 (Broek et al., 2008). 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 (Adeniyi and Ayepola, 2008). Plants are considered as largely complicated chemicals 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 (Sani *et al.*,2015).

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, Tasinania 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 (Adeniyi and Ayepola, 2008). 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 (Badrunisa *et al.,* 2011).Eucalyptus fresh leaves have eucalyptol (1,8 sineole) as the active ingredient and this is responsible for its various pharmacological actions (Adeniyi and Ayepola, 2008). 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 fleshly leaves of aloe vera plants contain not only cell wall carbohydrates such as cellulose and hemi cellulose but also storage carbohydrates such as

acetylated mannans (Negatzadeh-Brandozis, 2013). 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 (Agarry *et al.,* 2005). *Cocos nucifera* (coconut) belongs to the family *Aracaceae*. The plant is mainly used as a staple food crop and a source of wood and handcrafts, 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 (Taiwo *et al.,* 2011) Esquenazi *et al.,(*2011) in his studies reported that in 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 (Thormar, 1996).

Ginger (*Zingiber officcinale*) 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 (Islam *et al.,* 2014).

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 (Yaegaki and Jeffrey, 2000). Halitosis is detrimental to once 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 (Taiwo *et al.,* 2011). 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*, *Staphylococu aureus*, *Pseudomonas aeruginosa and klebsiella pneumonea),*
- to inhibit the growth of micro organism causing halitosis,
- to suppress volatile sulphide compound in the mouth.

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, *Staphylococus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonea*.

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, Stapyylococus aureas, 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. 50 g of the crushed sample was subjected to soxhlet extraction using 400 ml 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 (Ashwari and Sahu, 2014).

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 (Sarah, 2012).

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 (Adeniyi *et al.,* 2014).

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 (Renisheya *et al.,* 2014).

Production of the Mouthwash

The whole extract (ginger paste, aloe vera gel, eucalyptus extract and coconut oil) were mixed in different proportions (Sean, 2015).

Composition of the mouthwash:

Each 1000 ml of the mouthwash contains

Ginger paste 20 ml

Aloe vera gel 7 ml

Eucalyptus extracts	15.5 g
Coconut oil	15 ml
Ethanol	1.5 ml
Baking soda	1g
Distilled water	957 ml

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 Lashkari *et al.,* (2010). 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 30-80 ul 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 6:30 a.m. 24 students of Science Laboratory Technology of Federal Polytechnic Idah aged 20-25 years were selected and grouped into four, (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 clorsum was scraped with cotton wool and the scraped material was smelled after 20 seconds. Organoliptic 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) (Cees *et al.,* 2014). 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 above was repeated by the two examiners and result was recorded.

RESULT AND DISCUSSION

Table 1 and 2 shows the results of test obtained by the two examiners before the administration 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 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)
Group C	0	Absence of malodour	0(0%)
	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)

Table4: 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)

Table5: 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 = *Staphyloccus aureus* P.A = *Pseudomonas aeruginosa*, K.P = *Klebsiella pneumonia,* 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 1used 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%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%0 has moderate and severe malodour. The second examiner used spoontest 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%0 absence, questionable malodour. Group D, 2(33%) absence malodour, questionable malodour and strong malodour while nobody 0(0%0) has slight malodour, moderate and severe malodour.

Notice: there was 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 showed 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, Staphyloccus aureaus, 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 aureas*, 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 (Babayi et al., 2004). 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.

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 the Microbiology laboratory, Department of Science Laboratory Technology, Federal Polytechnic Idah is acknowledged.

REFERENCES

Adeniyi, B.A and Ayepola, O.O.(2008). The antibacterial activity of leaf extracts of *EUCALYPTUS Camaldulensis (Myrtaceae). Journal of applied sciences research*, **4**(11):1410-1413.

Adeniyi, O.P., Sanusi, R.A. and Obatolu, V.A.(2014). Effect of raw and cooked ginger (*Zingiber officinale*) extracts on serum insulin in normal and diabetic rats. *International Journal of Clinical Nutrition,* **2**(4):69-73.

Adewole, R.A., Eweka, O.M. and Agbelusi, G.A. (2014). A review of knowledge, diagnosis and treatment of halitosis among Nigerian dentist- a pilot study. *Herbert open access Journals,* **10**:2- 6.

Agarry, O.O, Olaleye, M.T. and Bello-Micheal,C.O. (2005). Comparative anticrobial activities of aloe vera gel and leaf. *African Journal of Biotechnology*, **4**(12): 1413-1414.

Akinyele, T.A, okoh, O.O, Akinpelu, D.A. and Okoh A.F. (2011). In-vitro antibacterial properties of crude acqueous and n-Hexane extracts of the husk of *Cocos nucifera. Molecules,* **16**:2135-2145.

Ashawani, K.D. and Sahu, O. (2014). Extraction of Eucalyptus oil as reduction of bacteria growth in driking water. *International Letters of Natural Sciences*, **16**: 121- 130

Babayi, H., Kolo, I., Okogunu, J.I. and Ijah, U.J.J. (2004). The antimicrobial activities of methanolic extracts of *Eucaluptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemidtri*, **16**: 106 – 111

Badrunisa, S., Vinita, R.P. and Shantura, M. (2011). Antibacterial activity of *Eucalyptus tereticornis* extracts for in use coolants of steel industry. *International Journal of Research in pharmaceutical and biomedical sciences*, **2**(4):1789-1794.

Broek, W.V.D, Feenstra, L and Baat, C. (2008). A review of the current literature on management of halitosis. *Oral diseases,* **14**:30-39.

Cees De. B, Jan, M, Broek, V.D, Annemick, M.WT, and Louw, F. (2014). Diagnosis of halitosis complaint, by multidisciplinary Team. *ONDM*, **13**(2):348-353.

Crispian, S. and Greenman, J. (2008). Halitosis (breath odor). *Peridontology,* **48**:66-75

Esquenazi, D., Wigg, M.D., Miranda, M.M., Tostes, J.B, Rozental, S., da Silva, A.J.R, Alviano, C.S. (2002). Antimicrobial antiviral activities of polyphenolics from *Cocos nucifera linn.* (Palmae) husk fiber extract. *Res.microbial,* **153**:647-652

Islam, K, Rowsni, A. A, Md.Khan, M and Kabis, S. (2014). Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria. *International Journal of science, environment,* **3**(3); 867-871.

Jose, R.C, Barbosa, M. D. S and Westphal, M. A. (2008). Halitosis: a review of associated facts and therapeutic approach. *Braz oral RCS*, **22**(1): 44-54.

Lashkari, P. D., Patel, R. K. and Modu, V. (2010). Antimicrobial activity of herbal mouthwash-Arowash liquid. *International chem. Tech Research*, **2**(1):99-101.

Negatzadeh-Brandozis, F. (2013). Antimicrobial activities and antioxidant capacity of aloe vera. *Negatzadeh-Barandozi organic and medicinal chemistry leters*, **3**:5.

Renishega, J. J. M. T., Johnson, M., Nancy, B.S., Laju, R. R., Arupriya G and Renola,J.J.T. (2014). Antibacterial and Antifungal activity of Aloe vera. *International Journal of Biomedical and Advance Research*, **3**(3) 184-187.

Sarah. (2012). Make your own virgin cold pressed coconut oil food recipes.

Sani, I., Abdulhamid, A. and Bello, F. (2014). Eucalyptus Camaldulensis phytochemical composition of ethanoic and acquos extracts of the leaves, stem-back, root, fruits and seeds. *Journal of scientific and innovative Research*.

Sean, N.(2015). 9 best homemade natural mouthwash recipe.

Taiwo, A.A., Omobola, O.O., David, A.A. and Anthony, I.O.O. (2011). In-vitro Antibacterial Properties of crude equeous and n-hexane extracts of the husk of *Cocos nucifera. Molecules*, **16**:2135-2145.

Thorma, P.K. (1996). *Hand book on coconut palm*. Oxford and IBU publishing co. New Delhi, India.

Yaegaki, K. and Jeffrey, M.C. (2000). Examination, classification and treatment of halitosis:L clinical properties. *Journal of the candian Dental Association*, **66**(5): 257-261.

Reference to this paper should be made as follows: Fatai Abiola Lateef et al., (2018), Evaluation of Some Selected Plant Extracts against Halitosis Causing Organisms *J. of Medical and Applied Biosciences,* Vol. 10, No. 1, Pp. 31-57