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Evaluation of Phytochemical and Antibacterial Potential of *Vernonia amygdalina* and *Elaeis guineensis* on Some Bacteria Associated with Diarrhea

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Bitter leaf and palm oil combination in treating diarrhoea is common in Africa. The aim of this study is to determine the synergistic and antimicrobial potential of *Vernonia amygdalina* and *Elaeis guineensis* on bacteria isolates from diarrheic stool.
Methodology: One hundred and fifty (150) diarrheic stool samples from the Diagnostic Laboratory in Rivers State University Teaching Hospital (RSUTH), Port Harcourt, were inoculated in Selenite F broth, MacConkey, Nutrient and Salmonella-Shigella agar plates using standard methods. Fresh and dry bitter leaf extracts were prepared by mashing, weighing and dissolving 100 grams(g) and 150 g concentrations of each in 100 milliliters(mls) of ethanol and sterile distilled water respectively. Undiluted mixture of bitter leaf and palm oil were prepared by mixing 2 mls of each in a sterile beaker. Zero-point one milliliter (0.1ml) of each undiluted extract, their mixture, ethanol and palm oil were separately dispensed into appropriately labeled wells using sterile pipette for sensitivity test on the isolated bacteria. The pH, temperatures and the phytochemical contents of the bitter extracts and palm oil were determined using pH meter, thermometer and spectrophotometric method.
Results: *Escherichia coli, Staphylococcus aureus and Salmonella sp.* were isolated. The pH and temperature of bitter leaf extract and palm oil were 7.2 and 26^0C and 6.7 and 26^0C respectively while the pH and temperature for bitter leaf and the palm oil mixture was 6.9 and 26^0C. The bitter

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leaf and palm oil extracts, their combination, ethanol and distilled water used on the isolates as antibiotics did not show any sensitivity. The phytochemicals identified in the bitter leaf were saponins, alkaloids and tannins while carotene was detected in the palm oil.
Conclusion: It can be concluded that the bitter leaf extract, palm oil and their combination did not inhibit the growth of bacteria as a result of lack of bioactive components from the bitter leaf. The nature of the diluents used for the extraction may not have been the right one as it would have caused the low concentration, dilution and disfiguring of the molecules of the bioactive compounds that prevented the proper reaction between the bacteria and the extracts used.

Keywords: Bacteria; extracts; diarrhoea; bitter leaf; palm oil.

1. INTRODUCTION

Plant extracts are continuously being sought for as effective and cheaper alternative sources of medication all over the world. The use of plant extracts in the treatment of human ailment is an ancient art, a practice that has passed on from generation to generation. Research into traditional plants received further boost due to the increasing resistance to many orthodox medications and thus, a search for new organic molecules from plants with antimicrobial properties [1]. Bitter leaf is a small tree or a shrub belonging to the family of *Asteraceae,* and it is a popular vegetable in Africa which grows in several parts of subtropical and tropical Africa [2]. It is known as “Ewuro” in Yoruba, “Onugbu” in Igbo, “Chusar-doki” in Hausa, “Oriwo” in Edo, “Etidot” in Ibibo, “Ityuna” in Tiv, and ‘Grawa” in Amharic. In other parts of Africa, it is also known as *Ndole* or *Muop (*Cameroon), *Tuntwono* (Tanzania), and *Mululuza* (Uganda) [3]. Palm oil is always extracted from two types of oil palm fruit: *Elaeis guineensis,* which is common in African and *Elaeis oleifera*, which is found in South America. Historical accounts suggest that palm oil was a part of the diet of indigenous populations. Presently, it has become the second most traded oil crop in the world, after soy, with Malaysia and Indonesia as its main producers. Palm oil is not to be confused with palm kernel oil. Both are obtained from the fruit, but the palm kernel oil is gotten from the seeds. Palm kernel oil has a higher amount of saturated fat, which makes it ideal for cooking [4]. Palm oil, also called red oil, contains high amount of saturated fat, antioxidants and vitamins, and is used as cooking oil, its stability makes it easier to be stored at room temperature for many months. It is also used as an ingredient in soups and sauces, dietary supplement, personal care and household products, skin moisturizer, sun block and biofuel.

Diarrhea is an abnormal increase in the frequency of emptying waste matter from the bowel. It is characterized by loose, watery stools, and usually last a few days [5]. Signs of dehydration often begin with loss of the normal stretchiness of the skin and irritable behavior. This can progress to decreased urination, loss of skin colour, fast heart rate, and a decrease in responsiveness as it becomes more severe. Diarrhea can be caused by viruses, bacteria or parasite, a condition also known as gastroenteritis. *Campylobacter sp* are a common cause of bacterial diarrhea, but infections by *Escherichia coli, Salmonella sp, Shigella sp and Staphylococcus aureus* are also a frequent cause. These infections are often acquired from water or food that has been contaminated by faeces. The presence of resistant genes which results in multi drug resistance in diarrhoea treatment calls [6,7] for other treatment options of diarrhoea caused by microorganisms. Phytochemicals are the active biological component of plants which include alkaloids, tannins, terpenoids and flavonoids [8]. Some plant extracts inhibit peptidoglycan synthesis, damage microbial membrane structures, modify surface hydrophobicity and modulate quorum-sensing [8]. This research is designed to determine the synergistic and antibacterial effect of bitter leaf (*Veronia amygdalina* delile) and palm oil (*Elaeis guineensis* Jacq*)* on some organisms associated with diarrhea.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Fresh leaf sample of bitter leaf and palm fruits were purchased from Mile 3 General Market, Diobu in Port Harcourt, Nigeria. The plant and palm fruits were identified by a Plant Taxonomist in the Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University, Port Harcourt.

2.2 Preparation of Plant Extracts

2.2.1 Fresh leaves extract

The fresh bitter leaf plant was used to prepare three extracts. The leaf sample was rinsed three

times with clean water. The fresh leaves were then mashed using sterile mortar and pestle in Medical Microbiology Laboratory, Faculty of Science, Rivers State University, Port Harcourt. Some portions of the leaf were shade-dried for few days and ground with a sterile surface grinder into powdery form and stored in clean tight-capped bottle, labeled and dated [9]. One hundred gram and one hundred and fifty grams (100 g and 150 g) of each of the ground fresh leaves were weighed in three batches. The first batch was soaked in 100 mls of ethanol, stoppered with cotton wool, covered with aluminum foil and left to stand for 24 hours in the refrigerator. This was then filtered using Whatman's No 1 filter paper and the extract stored in the refrigerator. The second batch of 100 g and 150 g of ground fresh bitter leaf were soaked in 100 mls of water, stoppered with cotton wool, covered with aluminum foil and left to stand for 24 hourrs in the refrigerator. This was filtered using Whatman's No 1 filter and stored in the refrigerator. The third batch of the 100 g and 150 g of the ground fresh bitter leaf were placed in a sterile muslin cloth respectively and the direct liquid was squeezed out from them individually into separate clean sterile containers. These were filtered with Whatman's No 1 filter paper and decanted into a sterile bottle that were properly labelled and stored in the refrigerator for use [9].

2.2.2 Dry leaves extracts

One hundred grams (100 g) and 150 g separately of ground powder of the dry bitter leaf were each weighed and placed into 4 separate sterile conical flasks of 250 ml capacity to be used for ethanol and water extractions separately. One hundred milliliter (100 mls) of absolute ethanol and water were separately measured and added to each conical flask that has been properly labeled respectively. These were shaken properly as the mixtures were left to stand at room temperature for 24 hours. Each was then filtered by passing it through a muslin cloth and then filtered with Whatman's No 1 filter paper with each filtrate stored in the refrigerator for further use [9].

2.2.3 Palm oil extraction

The Palm fruits were washed thoroughly in clean water and boiled to soften the skin. It was then mashed in a mortar to separate the pulp from the nuts. The whole mass was immersed in water, stirred and the palm oil was skimmed off. The fiber was sifted out of the water and the nuts were collected and separated from the remaining fiber. The palm oil obtained was then boiled and the palm oil was decanted into a sterilize reagent bottle and kept in the refrigerator for further use [10].

2.3 Collection and Processing of Samples

Stool samples were collected from patients presenting with gastroenteritis at the Rivers State University Teaching Hospital (RSUTH), Port Harcourt. A total of 150 samples were processed for pathogenic bacteria, using standard protocols of stool bacteriology as given by Chesbrough [10]. All identified bacteria were tested for antimicrobial susceptibility using the different obtained extracts.

2.4 Bacterial Sensitivity Testing Using Agar Well Diffusion Technique

The fresh and dried leaf extracts of bitter leaf were subjected to bacterial sensitivity test. Two dried agar plates that were properly labelled were used for the testing, one for the prepared fresh leaves and the other for the prepared dried leaves. One milliliter(1.0 ml) of each was dispensed onto the dried surface of a sterile nutrient agar plate. They were evenly distributed and allowed to dry but properly covered to avoid atmospheric contamination. The plates were bored with a sterile cork borer of about 8 mm. Zero-point one milliliter (O.1 ml) of each extract from the fresh and dried bitter leaf of different concentrations were aseptically dispensed into the wells of about 8 mm deep including both positive and negative control (synthetic agent and ethanol) [11,12]. The wells were labelled as follows Well 1 – Ethanol alone, Well 2 – Palm oil alone, Well 3 – Water extract of fresh bitter leaf, Well 4 - Water extract of dry bitter leaf, Well 5 – Fresh bitter leaf liquid, Well 6 – Ethanol extract of fresh bitter leaf, Well 7- Ethanol extract of dry bitter leaf and Well 8 – mixture of fresh bitter leaf liquid and palm oil. In well 8, 2 mls of the bitter leaf crude extract and palm oil were mixed together and 0.1 ml of the mixture was dispensed into the well. Ciprofloxacin, an orthodox antimicrobial agent was used as a positive control. The plates were then incubated for 24 hours at 37ºC.The sensitivity of the extracts and the control were observed [12].

2.5 Phytochemical Screening

Phytochemical screening was performed on the crude extract of bitter leaf for detection of

alkaloids (Dragendorff test), flavonoids (Shinoda's test), tannins (lead acetate test), saponins (Rothing test), glycosides, (Liebermann-Burchard test) and carotenes. These were carried out using standard methods as described by Ehiowenwengeon et al [13].

2.6 Determination of pH and Temperature of Bitter Leaf and Palm Oil and their Mixture

2.6.1 pH

With the aid of small pH pocket sized pH meter (Made by Hanna Instrument). A standard buffer solution of known pH of 4 and 7 was used to calibrate the instrument after which the pH of the sample was taken.

2.6.2 Temperature

A mercury thermometer was placed into the sample and allowed to stay for five minutes for the stability of the temperature after which the reading was taken.

3. RESULTS

The inhibitory effect of ethanol and water extracts of bitterleaf isolated from diarrheic stool samples is represented in table 1 while table 2 shows the inhibitory effect of direct bitter leaf liquid and palm oil on bacteria isolated from diarrheic stool samples.

Table 3 on the other hand is used to represent the quantitative screening of active phytochemical compounds of bitter leaf extract and palm oil and Table 4 represents the mean value of pH and temperature of bitter leaf, palm oil and their mixture.

4. DISCUSSION

The effects of bitter leaf and palm oil on *Escherichia coli***,** *Salmonella sp,* and *Staphylococcus aureus* were investigated. Out of the 150 diarrheic stool samples obtained from RSUTH, 30 bacteria were isolated. Eleven (11) samples yielded *Escherichia coli,* whereas 8 samples yielded *Salmonella sp* while *Staphylococcus aureus* were isolated from eleven 11 samples. The research work revealed that *Escherichia coli***,** *Salmonella sp* and *Staphylococcus aureus* were not susceptible to either of the extracts as shown in Table 1. The result obtained in the work agrees with the observation of Eloff [14], who reported that the aqueous extracts of bitter leaf showed no inhibition on *Escherichia coli, Salmonella sp* and *Staphylococcus aureus*. He also reported that the most active ingredients in bitter leaf are not

Table 1. The inhibitory effect of ethanol and water extracts of bitter leaf on bacteria isolated from diarrheic stool samples

Extracts /Antibiotic	Bacterial Isolates	Concentration (g/ml)	
		100 g/100 ml	150 g/100 ml
Ethanol (fresh leaves)	*Escherichia coli*	Nil	Nil
	Salmonella sp	Nil	Nil
	Staphylococcus aureus	Nil	Nil
Water (fresh leaves)	*Escherichia coli*	Nil	Nil
	Salmonella sp	Nil	Nil
	Staphylococcus aureus	Nil	Nil
Ethanol (dry leaves)	*Escherichia coli*	Nil	Nil
	Salmonella sp	Nil	Nil
	Staphylococcus aureus	Nil	Nil
Water (dry leaves)	*Escherichia coli*	Nil	Nil
	Salmonella sp	Nil	Nil
	Staphylococcus aureus	Nil	Nil
Ciprofloxacin (control)	*Escherichia coli*	S	S
	Salmonella sp	S	S
	Staphylococcus aureus	S	S

Nil = No Inhibition, S = Sensitive

Table 2. The inhibitory effect of direct bitter leaf liquid and palm oil on bacteria isolated from diarrheic stool samples

Extracts /Antibiotic	Bacterial Isolates	Concentration (g/ml)
		Neat
Fresh Palm Oil	*Escherichia coli*	Nil
	Salmonella sp	Nil
	Staphylococcus aureus	Nil
Fresh Bitter Leaf and Palm oil Combination	*Escherichia coli*	Nil
	Salmonella sp	Nil
	Staphylococcus aureus	Nil
Raw Bitter Leaf Liquid	*Escherichia coli*	Nil
	Salmonella sp	Nil
	Staphylococcus aureus	Nil
Ciprofloxacin (Control)	*Escherichia coli*	S
	Salmonella sp	S
	Staphylococcus aureus	S

Key: Nil= No inhibition, S= Sensitive

Table 3. Qualitative screening of active phytochemical components of bitter leaf extract and palm oil

	Active Components						
Extracts	**Saponins**	**Alkaloid**	**Tannins**	**Steroids**	**Flavonoid**	**Phenols**	**Carotenes**
Ethanol (fresh leaves)	**+**	**+**	**+**	Nil	Nil	Nil	Nil
Water (fresh leaves)	**+**	**+**	**++**	Nil	Nil	Nil	Nil
Ethanol (dry leaves)	Nil	**+**	**+**	Nil	Nil	Nil	Nil
Water (dry leaves)	Nil	**+**	**+**	Nil	Nil	Nil	Nil
Raw Bitter leaf(fresh)	+	+	+	Nil	Nil	Nil	Nil
Raw Bitter leaf (dry)	+	+	Nil	Nil	Nil	Nil	Nil
Palm Oil	Nil	Nil	Nil	Nil	Nil	Nil	+
	Control						
Ethanol	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Water	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Key: Nil = Not detected, + = Moderately detected, ++ = Absolutely detected

Table 4. Mean value of pH and temperature of bitter leaf, palm oil and their mixture

S/No	Sample	Parameters	
		pH	Temperature (^{O}C)
1	Butterleaf	7.2	26
2.	Palm oil	6.7	26
3.	Butterleaf and palm oil mixture	6.9	26

water soluble. Eloff [14] examined a variety of extracts for the ability to solubilize antimicrobial agents from plants and observed that the most commonly used solvent (ethanol and methanol) may not demonstrate the greater sensitivity in yielding antimicrobial chemicals on an initial screening.

According to Ibrahim et al. [15], the antibacterial susceptibility test of *Vernonia amygdalina* and

Elaeis guineensis showed that the aqueous extract of the plants showed low inhibition on food borne pathogens such as *Escherichia coli***,** *Bacillus cereus, Shigella dysentriae* and *Salmonella typhimurium* as shown in Table 2*.* Ali et al., [16] revealed that the ethanolic and aqueous extracts of bitter leaf were inactive against diarrhea causing organism such as *Escherichia coli***,** *Salmonellae sp* and *Shigella sp.* Evbuomwan et al*.,* [17] investigated the antibacterial activity of *Vernonia amygdalina* leaf extracts against multidrug resistant bacterial isolates. He reported that *Escherichia coli* was observed to be completely resistance to all concentrations of the plant extracts.

Uzoigwe and Agwa [18] carried out a research on the antimicrobial activity of *Vernonia amygdalina* on selected urinary tract pathogens. In their study, the isolates were *Klebsiella sp*, *Escherichia coli* and *Staphylococcus sp.* The bacterial isolate that showed different levels of susceptibility to both leaf and stem extracts of *Vernonia amygdalina* was *Klebsiella sp* while *Staphylococcus sp* and *Escherichia coli* were not susceptible to either of the extracts. According to Simplice et al., [19], 3 Gram negative bacteria; *Escherichia coli, Klebsiella pneumonia* and *Acinetobacter sp* were tested for their susceptibility to some antibiotics, the highest resistance rates were recorded with *Escherichia coli* been the highest at 76.6%. The obtained values from the determination of the pH and temperature of the extracts showed that the pH ranged from 6.9 to 7.2 whereas the temperature of the extracts was 26^{O} as is indicated on Table 4. These are not extreme pH that might prevent the growth of bacteria. This is because the isolated bacteria can grow at that pH since they are neutrophiles. The temperature of the different extracts was 26^{O}C and the incubation temperature for these bacteria was 37^{O}C. It could be that the temperature at which these extracts were introduced to the bacteria could have brought a sudden shock on the bacteria thereby making the bacteria cells to shrink and caused no reaction between the cell and the plant extracts. The determination of phytochemicals agrees with the work of Ojinnaka et al., [20] that supported the presence of saponins, tannins, and alkaloids to be present in *Vernonia amygdalina*. The method used in the extraction of the bioactive ingredient was mashing and extracting with water and ethanol which did not produce enough of the ingredients which agrees with the work of Ojinnaka et al.,[20], which stated that blanching and abrasive washing reduces the level of nutrients in the plant extract and low amounts of these nutrients, such as tannins, saponins and alkaloids were observed.

5. CONCLUSION

This study has shown that the aqueous, ethanol and direct bitter leaf liquid and palm oil were not effective on the bacterial isolates used. The bacterial isolates were susceptible to the ciprofloxacin antibiotics disc compare to the extract that showed no effectiveness against *Escherichia coli, Salmonella Sp* and *Staphylococcus aureus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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