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Antibacteria Assesement of *Aframomum melegueta* [roscoe] k. Schum [alligator pepper] Fruit on Bacteria Pathogen Isolated from the Urinary Tract

T. F. Abodunrin^{1*}, M. K. Oladumoye¹, F. T. Borishade¹, E. B. Akande¹ and O. I. Afolami¹

¹Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author MKO designed the study. Authors MKO and TFA performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FTB and IOA managed the analyses of the study. Author EBA managed the literature searche. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This study aims at assessing the Antibacterial activity of *Aframomum melegueta* against Urinary Tract bacteria. The study was carried out at the Microbiology department laboratory of the Federal University of technology, Akure, Ondo state, Nigeria, between February and June, 2018. Agar diffusion method was used in the susceptibility test, while tube dilution method was used determination of Minimum Inhibitory concentration. The phytochemical analysis showed the presence of alkaloids, flavinoids, saponins and tannins in both the Methanol and Pet-ether fruit extract, the result also showed that the concentration of all these compounds are higher in the Methanol extract than in the Pet-ether extract. The *in-vitro* Susceptibility test showed that *E. coli*, *P. mirabilis*, *S. aureus* were sensitive to methanol extract of *Aframomum. melegueta* at 100 mg/ml with *E. coli* showing the highest zone of Inhibition of 13.67 \pm 0.24 mm, while all the organisms were

^{*}Corresponding author: E-mail: towojuabodunrin@gmail.com;

resistant to Pet-ether extract at this concentration of 100 mg/ml except for *E. coli* with a zone of inhibition of 10.93 ± 0.07 mm at a highest concentration of 400 mg/ml. *S. aureus* was sensitive to Pet-ether showing a zone of inhibition of 16.97 ± 0.09 mm while *E. coli* had 20.33 ± 0.23 mm, *K. pneumoniae* and *P. aeruginosa* were resistant to both extract at all the tested concentration. Methanol Extract had MIC values of 50 mg/ml, 100 mg/ml and 50 mg/ml for *E. coli, P. mirabilis, S. aureus* respectively while the Pet-ether extract had an MIC values of 100 mg/ml and 200 mg/ml for *E. coli, and S. aureus* respectively. The MBC values for Methanol Extract were 100 mg/ml, 200 mg/ml and 50 mg/ml for *E. coli, P. mirabilis, S. aureus* respectively while the *Pet-ether extract* had an MIC values of 100 mg/ml and 200 mg/ml, 200 mg/ml and 50 mg/ml for *E. coli, P. mirabilis, S. aureus* respectively. The MBC values for Methanol Extract were 100 mg/ml, 200 mg/ml and 50 mg/ml for *E. coli, P. mirabilis, S. aureus* respectively while that of Pet-ether extract

Keywords: Aframomum melegueta; urinary tract; bacteria; antibacterial; pathogern.

1. INTRODUCTION

Infectious diseases are the number one cause of death due to illnesses across the world and account for approximately one-half of all deaths countries in tropical. According to World Health Organization [WHO] report, about 15 million [>25%] of 57 million annual deaths worldwide are the direct result of infectious diseases [1]. Of these infectious diseases, microorganisms are the commonest organisms responsible for morbidity and mortality [2,3]. As such, bacterial and fungal diseases continue to remain a major public health problem [4]. Urinary tract infections [UTI] are one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients, and can lead to significant mortality UTIs account for more than 8 million visits to physicians' offices, 1.5 million emergency room visits, and 300,000 hospital admissions in the United States annually [5]. Typical symptoms associated with UTI include the triad of dysuria [painful urination], urgency [the enhanced desire to void the bladder] and frequency [increased rate of urination] [5].

Due to indiscriminate use of synthetic antimicrobial drugs, microorganisms resistant and or multi resistant to major class of antibiotics have emerged in recent years and the situation is exacerbated too [6,7]. Moreover, high cost and adverse side effects of popular synthetic antibiotics are major burning global issues [8]. To this regards, antibiotics resistance has resulted in morbidity and mortality while the high cost and adverse side effects have increased health care costs. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines [9,10]. In another words, increasing capability of microbes to develop multidrug resistance has no doubt encouraged search for new, safe and effective bioactive agents of plant origin considering the fact by previous authors [11], that traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents.

Medicinal plants have received huge attention both in the developed and developing nations. Their economic importance has drawn attention of various world bodies mostly; the World Health Organization [WHO] which released a special document concerning collection practices for medicinal plants [12,13]. The use of medicinal plants has always been part of human culture and is wide spread in Africa. In some countries, like Ghana, government encourages the use of indigenous forms of medicine rather than expensive imported drugs. Also in Nigeria, a large percentage of the populace depends on herbal medicines because the commercially available orthodox medicines are becoming increasingly expensive and out of reach [14]. Aframomum melegueta is a tropical herbaceous perennial plant of the genus Aframomum belonging to the family Zingiberaceae [ginger family] of the angiosperms in the Kingdom plantae. The seeds have pungent pepperv taste due to aromatic ketones [15,16]. It is a plant with both medicinal and nutritive values,found commonly in rain forest. It is widely spread across tropical Africa.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plants

Fresh whole seed pod of *Aframomum melegueta* were collected from Oja Oba in Akure south local Government, Akure, Ondo state. The plants were identified by the department of Agricultural science, Federal University of Technology, Akure, Ondo state. The fruit of *Aframomum melegueta* were then washed with distilled water to remove dirts, and then air-dried at room temperature for 21 days until it is well dried to be milled using an electric blender.

2.2 Collection and Identification of Isolates

Urinary Tract Isolates were obtained from the Microbiology laboratory of the Federal University of Technology, Akure, Ondo State. Identification of bacterial isolate was made on the basis of Gram reactions, morphology, biochemical characteristics and cultural characteristics. Gram staining was performed to differentiate the Gram positive and Gram negative organisms. Pure cultures of the isolates were then be inoculated on Nutrient agar [Lab M] slants in test tubes and stored at 4°C for further studies.

2.3 Standardization of Test Orgnainsms

A loopful of test organisms were inoculated into 5.0 ml of nutrient broth and incubated at 37° C for 24 hours. 0.2 ml from the 24 hours culture of the organisms was then dispensed into 20 ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 10^{-6} cfu/ml the turbidity of the broth was then compared with MacFarand Standard [17].

2.4 Preparation of Plant Extract

A 50 g of the blended materials [*Aframomum melegueta*] was soaked in two hundred and fifty millilitres [250 ml] of 90% ethanol in a sterile conical flasks and allowed to soak at ambient temperature for 72 hrs. With frequent agitation, this process was repeated in distilled water. It was then be filtered and the filtrate evaporated to dryness in a water bath at a controlled temperature of 40°C, the crude extract was stored in the refrigerator at a temperature of 4°C for further analysis [18].

2.5 Qualitative Assay for Phytochemicals

The methods described in [19,20] was adopted for the qualitative phytochemical assay conducted on the extracts.

2.6 Quantitative Assay for Phytochemicals

The methods described in [21,22] were adopted for the quantitative phytochemical assay conducted on the extracts.

2.7 *In-vitro* Antibacterial Screening of Crude Extracts

The Agar diffusion method was used, sterile nutrient agar plates was prepared and 1 ml of

the test organism was mixed properly with 19 ml of nutrient agar and each plate was then labelled appropriately and allowed to solidify. A sterile cork borer [7 mm] was used to bore three holes of equidistance from each other on each plate. Molten agar was used to seal the base of each hole after which different holes was filled with different concentrations of the various extracts. The plates were left on the bench for 30 minute to allow diffusion of the extracts before incubation at 37°C for 24 hours. The zones of clearance [Inhibition] produced around the holes after incubation were observed, measured and recorded appropriately [23].

2.8 Minimum Inhibitory Concentration (MIC)

The tube dilution method described by Ajaiyeoba et al. 2003 was adopted. The MIC was determined by serially diluting extracts. 5 ml of each of the dilution representing a known concentration of the extract was introduced into 5 ml of sterile nutrient broth in test tubes. The mixture was then inoculated with 0.1ml of the test of organism previously standardized to 10⁶ and then incubated at 37°C for 24 hours. The least concentration of the extract with no turbidity the minimum was taken as inhibitory concentration (MIC) [23].

2.9 Minimum Bactericidal Concentration (MBC)

This was an offshoot of the previously determined MIC. The MBC of the plants extracts were determined by sub-culturing from all the tubes that showed no turbidity from the MIC test into a sterile nutrient agar plate, the least concentration in which no growth is observed was taken as the MBC [23].

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical analysis

The results of this work showed that the extracts obtained from *Aframomum melegueta* using both polar [methanol] and non-polar [pet-ether] solvents are comparatively rich in phytonutrients such as alkaloids, flavonoids, saponins and tanins, Methanol extract contains carbohydrates which is absent in the Pet-ether extract as shown in Table 1.

Fig. 1 shows the quantitative phytochemical screening the result shows that methanol extract has higher concentration of flavonoids [481 mg/g], alkaloids [227.5 mg/g], saponins [40.62 mg/g] and tanins [34.25 mg/g] as compared to the pet-ether extract which contains 105.8 mg/g, 78 mg/g, 32.1 mg/g, and 15.25 mg/g respectively. The result have also shown that polar solvent [Methanol] is a better extracting solvent compared to non-polar solvent [petether], Invariably, the high contents of the phytonutrient such as Alkaloids and Flavonoids of Methanol extracts compared to to petroleum ether extract can be directly attributed to the solubility index of the extraction solvents used thereby causing hydrophilic compounds in the plant to be liberated easily [24,25] this findings is also similar to reports by authors such as [26, 27,28].

The antibacterial activity of both Methanol and pet-ether was tested against 5 organisms (*Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Proteus mirabilis*) at concentrations of 100 mg/ml, 200 mg/ml, 300 mg/ml and 400 ml/ml. At a concentration of 100 mg/ml *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus* were susceptible to

methanol extract showing a zone of inhibition of 13.67 ± 0.24 , 10.50 ± 0.26 and 11.47 ± 0.24 respectively although there was no significant difference between the zone of inhibition shown by Proteus mirabilis and Staphylococcus aureus all the organisms resisted the pet-ether extract with the exception of Escherichia coli showing a zone of inhibition of 10.93 ± 0.07 at these concentration the positive control [ciprofloxacin] was observed to exert a higher zone of inhibition of 24.77 ± 0.23, 19.63 ± 0.27, 16.50 ± 0.26, 25.73± 0.13, 12.33 ± 0.28 on Escherichia coli, K. pneumonia, P. aeruginosa, Proteus mirabilis and Staphylococcus aureus respectively, at the highest concentration of 400mg/ml were still resistant to both extract, while were sensitive to the methanol extract of A. melegueta showing an increased zone of inhibition of 25.33 ± 0.23, 16.17± 0.18 and 20.27 ± 0.27 respectively with significant difference in the zone of inhibition recorded. while Escherichia coli and Staphylococcus aureus were aslo sensitive to pet-ether extract of A. melegueta showing a zone of inhibition of 20.33 ± 0.23 and 16.97± 0.09 respectively summarily K. pneumonia and P. aeruginosa resisted both extract at all the concentration tested, while Escherichia coli, Proteus mirabilis and Staphylococcus aureus

Table 1. Qualitative phytochemical analysis of Aframomum melegueta fruit

Compounds	Methanol extract	Pet-ether
Alkaloids	++	+
Flavinoids	+++	+
Saponins	++	+
Tannins	+	+
Phlobatanins		
Resin		
Carbohydrate	+	



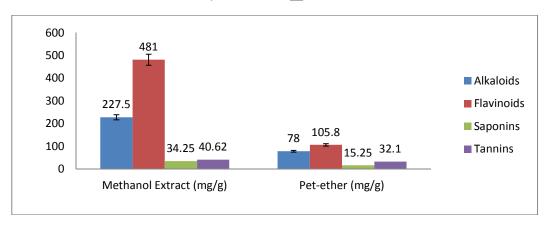


Fig. 1. Quantitative phytochemical analysis of Aframomum melegueta fruit

Pathogen	Zones of inhibition (diameter in mm)			
	Methanol extract	Pet-ether extract	Positive control (CIP)	Negative control (DMSO)
E. coli	13.67±0.24 ^c	10.93±0.07 ^b	24.77±0.23 ^c	0.00±00 ^a
K. pneumoniae	0.00±00 ^a	0.00±00 ^a	19.63±0.27 ^b	0.00 ± 00^{a}
P. aeruginosa	0.00±00 ^a	0.00±00 ^a	16.50±0.26 ^a	0.00 ± 00^{a}
P. Mirabilis	10.50±0.26 ^b	0.00±00 ^a	25.73±0.13 ^d	0.00±00 ^a
S. aureus	11.47±0.24 ^b	0.00±00 ^a	19.33±0.28 ^b	0.00±00 ^a

Data are presented as Mean±S.E [n=3]. Values with the same superscript letter[s] along the same column are not significantly different [P<0.05] Key: CIP: Ciprofloxacin; DMSO: Dimethylsulfoxide

Pathogen	Zones of inhibition (diameter in mm)			
-	Methanol extract	Pet-ether extract	Positive Control (CIP)	Negative Control (DMSO)
E. coli	16.93±0.07 ^d	13.87±0.13 ^c	24.77±0.23 ^c	0.00±00 ^a
K. pneumoniae	0.00±00 ^a	0.00±00 ^a	19.63±0.27 ^b	0.00±00 ^a
P. aeruginosa	0.00±00 ^a	0.00±00 ^a	16.50±0.26 ^ª	0.00±00 ^a
P. Mirabilis	12.00±0.00 ^b	0.00±00 ^a	25.73±0.13 ^d	0.00±00 ^a
S. aureus	13.70±0.30 ^c	11.83±0.17 ^b	19.33±0.28 ^b	0.00±00 ^a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05) Key:.; CIP: Ciprofloxacin; DMSO: Dimethylsulfoxide

Table 4. Antibacterial activity	y of A. <i>melegueta</i>	a Fruit extract at 300mg/ml on test isolates

Pathogen	Zones of inhibition (diameter in mm)			
	Methanol extract	Pet-ether extract	Positive Control (CIP)	Negative control (DMSO)
E. coli	19.99±0.03 ^d	16.77±0.23 ^b	24.77±0.23 ^c	0.00±00 ^a
K. pneumoniae	0.00±00 ^a	0.00±00 ^a	19.63±0.27 ^b	0.00±00 ^a
P. aeruginosa	0.00±00 ^a	0.00±00 ^a	16.50±0.26 ^ª	0.00±00 ^a
P. Mirabilis	13.23±0.23 ^b	0.00±00 ^a	25.73±0.13 ^d	0.00±00 ^a
S. aureus	16.00.0±00 ^c	16.63±0.23 ^b	19.33±0.28 ^b	0.00±00 ^a

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05) Key: CIP: Ciprofloxacin; DMSO: Dimethylsulfoxide

were sensitive to methanol extract at all the concentration tested similarly Escherichia coli was sensitive to pet-ether at all the concentration tested while Staphylococcus aureus was resistant at low concentration of 100mg/ml but sensitive to higher concentration. The Negative control DMSO showed no antimicrobial activity. The resistance of K. pneumonia and P. aeruginosa to the plant cannot be clearly ascertained by the scope of this work but There are three basic mechanisms by which organisms resist the action of antimicrobial agents: restricted uptake and efflux; drug inactivation and changes in targets. The outer membrane of P. aeruginosa is known to present a significant barrier to the penetration of antimicrobial agents [29], restricting the rate this could also be responsible for the resistance of the organism of penetration of small hydrophilic molecules and excluding larger molecules [29].

The MIC value of methanol extract for *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus* were 50 mg/ml, 100 mg/ml and 50 mg/ml while the value recorded for pet-ether were 100 mg/ml and 200 mg/ml for *Escherichia coli* and *Staphylococcus aureus*, the MBC value of methanol for *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus* 100 mg/ml, 200 mg/ml and 50 mg/ml respectively, this indicates that methanol extract is bactericidal at lower concentration on *Staphylococcus aureus* while it is bacteriostatic at lower concentration on *Escherichia coli*, *Proteus mirabilis*, pet-ether extract has an MBC value of 100 mg/ml on *Escherichia coli* but does

Pathogen	Zones of inhibition (diameter in mm)			
	Methanol extract	Pet-ether extract	Positive control (CIP)	Negative control (DMSO)
E. coli	25.33±0.23 ^d	20.33±0.23 ^c	24.77±0.23 ^c	0.00±00 ^a
K. pneumoniae	0.00±00 ^a	0.00 ± 00^{a}	19.63±0.27 ^b	0.00±00 ^a
P. aeruginosa	0.00±00 ^a	0.00 ± 00^{a}	16.50±0.26 ^ª	0.00±00 ^a
P. Mirabilis	16.17±0.18 [♭]	0.00 ± 00^{a}	25.73±0.13 ^d	0.00±00 ^a
S. aureus	20.27±0.27 ^c	16.97±0.09 ^b	19.33±0.28 ^b	0.00±00 ^a

 Table 5. Antibacterial activity of A. melegueta Fruit extract at 400 mg/ml on isolates from UTI samples

Data are presented as Mean±S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05) Key: CIP: Ciprofloxacin; DMSO: Dimethylsulfoxide

Table 6. Minimum inhibitory concentration [MIC] of the Aframomun melegueta extracts

Organisms	MIC values (mg/ml)	(mg/ml)
	Methanol extract	Pet-ether
Escherichia coli	50	100
Klebsiella pneumonia	NA	NA
Proteus mirabilis	100	NA
Pseudomonas	NA	NA
Staphylococcus aureus	50	200

Key: NA= No Activity

not exert bactericidal effect on *Staphylococcus* aureus.

Table 7. Minimum bactericidal concentration	n
[MBC] of the Aframomun melegueta extracts	5

Organisms	MBC values (mg/ml)		
	Methanol	Pet-ether	
	extract		
Escherichia coli	100	100	
Klebsiella	NA	NA	
pneumonia			
Proteus mirabilis	200	NA	
Pseudomonas	NA	NA	
Staphylococcus	50	NA	
aureus			

Key: NA= No Activity

The biological functions of many of these phytochemicals detected and quantified have been documented as findings from [28,27,25] revealed the pharmacologic effects exerted by flavonoids which include protection against allergies, inflammations, free radicals and tumors. Anosike et al. [26] reported that Flavonoids are known to be synthesized by plants in response to microbial infection. They have effective antimicrobial activities *in vitro* against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and also with bacterial cell, Schito [30] aslo reported the

pharmaceutical importance of tanins, it was reported that Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been attributed to tannins. Their mode of action is to complex with proteins through nonspecific forces, such as hydrogen bonding as well as by covalent bond formation. Thev also complex with polysaccharides which are components of bacterial cell wall. Studies show that tannins can be toxic to filamentous fungi, yeasts, and bacteria. Thus, the mode of antimicrobial action of this plant may be related to the ability of these bioactive constituents to inactivate microbial adhesins, enzymes, and envelope transport proteins. Alkaloids also are of therapeutic significance. Pure isolated alkaloids and the synthetic derivative are used as the basic medicinal agents due to their analgesic, antispasmodic and antibacterial potentials [31], Likewise, the presence of phenolic compounds as well as tannins in the extracts indicates their potential as antimicrobial agents [27,22,25]. The study have shown that the methanol extract possess more antimicrobial activity in comparison to the pet-ether extract this is most likely due to the presence of higher concentration of phytochemical constituent in the methanol extract as shown in table 1 this is also consistent with the work of Anosike et al. [26].

4. CONCLUSION

This research have further established the the use of the *Aframomum melegueta* fruit in traditional medicine. The work have shown that the fruit has potential of treating Urinary Tract infection, it has a broad spectrum of activity against both gram Positive and gram Negative Bacteria. Although further research on the plant is recommended to further purify the active compound and then carry out toxicity studies in a bid to further improve its activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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