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Phytochemical and Antimicrobial Activity Screening of Ether Extract of *Afromomum melegueta* Seed

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FUE and CNE conceptualized the research and sourced plant samples for the experiment, authors UTO and GCU carried out the laboratory work, author GCU also wrote the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The search for new antimicrobials as a result of antibiotic failure has led to the development of new antibiotic scaffolds. In the African sub-continent, this has translated to exploration of her rich flora for potential leads. This work investigated the antimicrobial potential of ether extract of *Afromomum melegueta* seeds against three candidate microbes- *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.
Methodology: Seeds of *A. melegueta* were extracted with petroleum ether using cold maceration technique; standardized chemical tests were employed for phytochemical screening and the agar-well diffusion method used for antimicrobial analysis of the obtained extract.
Results: Findings indicate that the extract displayed dose-dependent inhibitory effect on the growth of *E. coli, S. aureus* and *C. albicans* with maximum inhibition zones of 5, 10 and 3 mm at extract concentration of 25, 20 and 25%, respectively. The observed antimicrobial activity may be as a consequence of the phytochemistry of the seeds, as preliminary phytochemical screening revealed the seeds to contain saponin, flavonoid, cardiac glycoside, alkaloids, cyanogenic glycosides, tannin and phenolic compounds. The ability of the extract to inhibit microbes with diverse morphology indicates that the antimicrobial bioactive principles therein may have broad specificity.

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Conclusion: The result of our study further reinforces the importance of *A. melegueta* seeds in ethnomedical practices and its use in culinary. Further investigation is however needed to isolate and mechanistically investigate the bioactive antimicrobial principles for potential development into generic antimicrobials.

Keywords: Afromomum melegueta; E. coli; C. albicans; S. aureus; antimicrobial; seed; petroleum ether.

1. INTRODUCTION

It is now widely accepted that antimicrobials have limited clinical lifespan, as pathogenic microorganism evolve rendering heretofore potent antimicrobial agents ineffective [1]. This has led to an intensified effort geared towards the discovery/synthesis of new antimicrobial leads. Despite the intensified search, new discoveries do not always translate into new classes of approved pharmaceuticals. Over the past three decades for instance, only two novel classes of antibiotics have been launched on the market: the oxazolidinone linezolid and the cyclic lipopeptide daptomycin [2]. This small number of novel compounds demonstrates the inadequacy of traditional approaches, prompting the adoption of new strategies.

To this end, some strategies have been adopted by researchers in developing newer drugs. The use of bacteriophages [3], marine peptides [4], microbial polyketides [5], and secondary metabolites/peptides from plants [6–8], have yielded some of the more promising leads.

Researchers in Africa are especially paying attention to plants as source of new therapeutic leads for reasons which include; Africa's rich flora which makes the continent a hub for plants with medicinal potentials, and continuing dependence of a sizeable proportion of the African populace on herbs for the treatment of common infectious diseases [9,10]. Consequently, there's been a surge in research bordering on developing therapeutics, including antimicrobials, from plant bioresources.

Aframomum melegueta K. Schum, belongs to the ginger family (Zingiberaceae) and is colloquially called grains of paradise or alligator pepper. It is variously known locally as *ose oji in* Igbo*,* ataare *in* Yoruba *and cittáá* in Hausa of Nigeria [11].

The seed of *A. melegueta* is used in different African cultures as a spice, medicine or for other preternatural roles. In folk medicine, the seeds are employed as local remedy for stomach ache, snakebite, diarrhea, cardiovascular diseases, diabetes and inflammation [12]. The seeds are also used in preparing yam pottage for new mothers to enhance appetite and reduce the risk of puerperal infections in most parts of Southern Nigeria [13].

The aforementioned ethnomedical uses of *A. melegueta* have prompted scientific scrutiny into its therapeutic properties. Several studies have empirically highlighted the antimicrobial potential of different extracts of *A. melegueta* on a variety of microorganisms [14–19].

The present work intends to further add to the body of knowledge on the antimicrobial potential of *A. melegueta* seed by reporting for the first time, to the best of our knowledge, the effect of the petroleum ether extract of the seeds on three select microbes- *E. coli*, *S. aureus* and *C. albicans*.

2. MATERIALS AND METHODS

2.1 Plant Material Collection and Identification

Dry pods of *A. melegueta* were bought from Eke-Awka market, a popular vegetable and spice market in the capital city of Anambra State, Southeastern Nigeria. The seeds were identified by a taxonomist with the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria.

2.2 Extract Preparation

The pods were cut open and the seeds collected. Thereafter, seeds were pulverized using pestle and mortar to obtain a powdered form which was stored in airtight plastic containers until needed for the experiment. The powdered seed (200 g) was macerated in 1 L of petroleum ether (40-60°C) in an Erlenmeyer flask for 24h, and the mixture agitated mechanically at intervals. The mixture was filtered afterwards using Whatman No. 1 filter paper in a Buchner funnel. The filtrate obtained was concentrated in water bath (Chem-

index, WB500E, USA) at 70°C to obtain a gel-like concentrate. The concentrate obtained was stored in refrigerator at 4°C until needed for analysis.

2.3 Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out using standardized procedures. The tests are briefly described below:

2.3.1 Phenols

The Million's test was employed [20]. Two mL of extract was added to 3 mL of Million's reagent in a test tube and heated in a water bath for 2 min at 70°C. Pink-red colour formation indicates the presence of phenolic groups.

2.3.2 Flavonoids

Lead acetate test: To 1 ml of the filtrate, 1 ml of 10% lead acetate solution was added. Appearance of a buff-coloured precipitate indicates the presence of flavonoids [21].

Ferric Chloride test: To 1 mL of extract in a test tube, a few drops of 10% $FeCl_2$ was added. A green-blue or violet coloration indicates the presence of a phenolic hydroxyl group [21]

2.3.3 Alkaloids

One ml of the extract was mixed with 5 mL of 2% HCl in a test tube, heated on water bath, and filtered. Of the filtrate, 2 mL was divided into two aliquots of 1 ml each. To the first portion, few drops of Wagner's reagent were added; occurrence of reddish-brown precipitate is taken as a positive test. To the second aliquot, 1 ml of Mayer's reagent was added and appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [22].

2.3.4 Saponins

The extract (1 ml) was boiled with 5 ml of distilled water, the soluble fraction of the mixture was decanted into two aliquots while still hot. The obtained filtrate was used for the following tests:

Emulsion test: Two drops of olive oil was added to 1 ml of the extract in test tube. The set up was mechanically agitated and observed for formation of emulsion; which indicates the presence of saponin.

(Frothing test). Distilled water (3 mL) was added to 1 ml of the extract. 0.5 mL filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable froth head indicates the presence of saponin [23].

2.3.5 Cardiac Glycoside

The Salkowski test was employed for this. The extract (1 ml) was dissolved in 2 mL of chloroform. Sulphuric acid was then carefully added to form a lower layer. A reddish-brown colour at the interface indicates the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside)

2.3.6 Carbohydrates

Iodine test was employed. A few drops of iodine was added to 2 ml of the extract in a test tube. A blue-black colouration indicates the presence of starch.

2.3.7 Reducing Sugars

To 2 mL of the extract, a few drops of Fehling's solution A and B were added; an orange-red precipitate suggests the presence of reducing sugar.

2.3.8 Tannin

Ferric chloride test: A quantity of the extract (2 ml) was heated with 5 mL of water for 5 min in a test-tube. One mL of the filtrate was diluted with 5 mL distilled water in a test tube; few drops of 0.1% ferric chloride solution were added. A characteristic blue-black, green or blue-green coloured precipitate indicates the presence of tannin.

Bromine water: Bromine water (2 ml) was added to 2 ml of the extract. Presence of condensed tannin is indicated by greenish-red colouration.

2.3.9 Cyanogenic glycosides

Two ml of the extract was dispensed into a 100 mL conical flask containing 10 ml of water. Using a thread, a picrate paper was suspended into the flask and the flask's bore covered with a cork. The set-up was heated in a water bath for 1 h. Change in colour of the paper from yellow to brick red indicates the presence of cyanogenic glycosides.

2.4 Antimicrobial Studies

2.4.1 Collection of microbial samples

Pure microbial cultures of *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923) and *Candida albicans* (ATCC 10231) were obtained from Peace Laboratory and Diagnostic Centre Awka, Anambra State Nigeria, in agar slants.

2.4.2 Preparation of nutrient agar and extract

Dehydrated powder of Nutrient agar (Sigma-Aldrich, USA) with composition (gL^{-1}): meat extracts 1.0, yeast extract 2.0, peptone 5.0, sodium chloride 5.0, agar 15.0; pH 7.4 was used as the microbial culture media. It was prepared following manufacturers instruction by suspending 28 g in 1 L of distilled water, boiling the mixture to obtain a consistent liquid broth and subsequently sterilized by autoclaving at 121°C for 15 min.

Different concentrations of the extract; 10, 15, 20 and 25% (v/v) were prepared by dissolving 1, 1.5, 2 and 2.5 mL respectively, of the extract in 10 mL of distilled water.

2.4.3 Inoculation and incubation of agar

The prepared nutrient agar was poured into sterile, labeled 150 mm petri-dishes and allowed to set for 24 h. A sterile swab was dipped into the broth culture containing the microorganism, gently removed and used in streaking the surface of the nutrient agar to form a microbial lawn. For the agar well diffusion assay, 4 mm diameter holes were aseptically bored in the media plates using a sterilized cork borer, and 50 µL of various extract concentrations (10, 15, 20 and 25%) were introduced into the wells using a micropipette. The plates were incubated overnight at 37°C. Microbial growth inhibition was determined by measuring the diameter of the inhibition zones around the wells. The experiment was done three times and the mean values were presented.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Analysis

The result of the preliminary phytochemical study carried on petroleum ether extract of *A. melegueta* seeds to determine the presence of medicinally important phytochemicals in the seeds revealed the presence of various phytochemicals such as tannins, saponin, flavonoids, alkaloids, cardiac glycosides, and reducing sugars in the extract of *A. melegueta* seeds (Table 1).

3.2 Antimicrobial Studies

As the pharmacological action of any plant cannot be accurately ascertained by the result of preliminary phytochemical studies only, thus, the antimicrobial activity of the extract against select microbes was also evaluated. The present investigation showed the efficacy of the extracts in inhibiting the growth of *S. aureus* and *C. albicans* (Table 2).

Table 1. Qualitative phytochemical analysis of the petroleum ether extracts of *A. melegueta* seeds

SN	Phytochemical constituent	Type of test	Remark/ Inference
1	Phenolic groups	Million's method	+
2	Flavonoid	$FeCl_2$	+
		Lead acetate	+
3	Alkaloids	Wagner's reagent	+
		Mayer's reagent	+
4	Saponin	Emulsion	+
		Frothing	+
5	Cardiac glycosides	Huppert Salkowski's	+
6	Carbohydrate	Iodine test	+
7	Reducing compound	Fehling's reagents	-
8	Tannin	Bromine water	-
		Ferric chloride	+
9	Cyanogenic glycosides	Keller-killiani	+

Note: Present(+), Absent(-)

Table 2. Antimicrobial activities of petroleum ether extract of *A. melegueta* seeds

Microorganism	Conc. of extract (%)	Zone of inhibition (mm)
Escherichia coli		
	10	1.0
	15	2.0
	20	3.0
	25	5.0
Staphylococcus aureus		
	10	3.0
	15	5.0
	20	10.0
	25	4.0
Candida albicans		
	10	0.0
	15	1.0
	20	2.0
	25	3.0

Qualitative phytochemical studies revealed the presence of a rich mix of phytochemicals in the seeds of *A. melegueta*. The phytochemicals detected include phenols, flavonoids, alkaloids, saponin, cardiac glycosides, tannin and cyanogenic glycosides.

Plant phenols have been demonstrated to have strong antioxidant capabilities, useful in preventing stress-associated diseases such as cancer and diabetes [24,25]. More recently, studies on the dermatologic effect of phenolics have shown them to be effective in treating skin disorders and slowing ageing [26].

Flavonoids are ketone-containing secondary metabolites found in plants and fungus. Flavonoids such as myricetin, quercetin and morin have been extensively investigated and proven to possess anti-inflammatory, antimicrobial and anti-proliferative activities [27].

Alkaloids are bioactive molecules with nitrogen constituting a key component of their molecular architecture [28]. Amongst its many therapeutic roles, the antimicrobial properties of different types of alkaloids (furoquinolones, indole alkaloids, acridones) have come into focus more recently, with a plethora of studies reporting the efficacy of alkaloids against protozoan parasites [29].

Saponins, typically composed of hydrophobic aglycone and hydrophilic sugar moieties, are active biosurfactants that have several therapeutic applications including immunostimulatory, mulluscicidal, hypocholesterolemic, antimicrobial and anti-oxidant activities [30–32].

Cardiac glycosides, also known as cardiotonic steroids, generally contain a steroid-like structure and induces cardiotonic effect via selective inhibition of Na^+/K^+-ATPase. They are therapeutically employed in treating cardiac congestion and some types of arrhythmias (Calderón-Montaño et al., 2014). Recent studies also shown that they possess anticancer [33] and antiviral [34] potentials.

Cyanogenic glycosides are mostly phytotoxins that have limited therapeutic uses, but may induce toxic effects in humans and livestock upon ingestion [35].

Tannins are primarily polymerization products of flavan-3-ol (condensed tannin) or gallic acid moieties linked to a carbohydrate core (hydrolysable tannins), and have wide therapeutic properties including antiploriferative and antimicrobial activities [36].

Studies on the antimicrobial potential of tannins have gained traction in recent years; with generally promising results being reported. Tannins were found to be effective in inhibiting biofilm formation in *P. aeruginosa* [37], while ellagic acid and ethyl-gallate (both of them tannins) were found to be bactericidal against *K. pneumonia* and *S. aureus* [38].

The antimicrobial findings of the present study showed that the petroleum ether extract of *A. melegueta* effectively inhibited the growth of the three test microbes- *E. coli*, *S. aureus* and *C. albicans* in a concentration-dependent manner.

For *E. coli*, the extract showed maximum inhibition of 5mm at the highest concentration considered (25%). This results agrees with the findings of Doherty et al. [16], who reported that ethanol extract of *A. melegueta* seeds at 50 mg/mL was able to inhibit the growths of *Samonella spp, E. coli*, *Shigella spp* and *Klebsiella spp* with average zones of inhibition of 15, 20, 15 and 30 mm respectively.

Similarly, Alo et al. [14], using disc diffusion method, observed that methanol and ethanol extracts of *A. melegueta* seeds were potent in

inhibiting the growths of *E. coli*, and *S. typhi* with inhibition zones of 12 and 23 mm, respectively.

The extract used in the present experiment was able to inhibit the growth of *S. aureus* in a dose-dependent manner with maximum inhibition (10mm) occurring at 20% extract concentration (Table 2). At 25%, there was a reduction (4 mm) in the zone of inhibition signaling a negative feedback. In a similar study, Ngwoke et al. [19], concluded that labdane diterpenes isolated from the rhizomes of *A. melegueta* exhibited significant antibacterial activity against methicillin resistant *staphylococcus aureus* (MRSA), *E. coli* and *L. monocytogenes*, with some of the diterpenes showing more activity than generic antibiotics- ampicillin, gentamycin and vancomycin, employed in their experiment as control.

Notably, the extract in the present experiment was able to inhibit the growth of *C. albicans*, a recalcitrant fungal pathogen notorious for being resistant to several antibiotic classes. However, the extract was less effective in inhibiting the growth of *C. albicans* compared to the bacteria strains tested, with maximum inhibition of 3 mm occurring at extract concentration of 25%. This finding corroborates the work of Chiejina and Ukeh [15], who reported methanolic extract of *A. melegueta* seeds to possess antifungal activity against screened pathogenic fungi, including *C. albicans* and *A. niger*.

Definitely, the antimicrobial properties noted in our finding and other related works must be connected to the rich pytochemistry of the seeds as shown by the result of the phytochemical screening as several of the detected secondary metabolites have been empirically demonstrated to have antimicrobial/chemotherapeutic abilities.

4. CONCLUSION

Clearly, plant could serve as veritable sources of newer antimicrobial leads, and research in this field should to vigorously pursued as new antibiotics (or their scaffolds) are urgently needed if the current antimicrobial resistance menace is to be overcome.

Interestingly, *A. melegueta*, renowned for its many ethnomedical and culinary uses has been widely investigated for antimicrobial potentials, with most of the results showing promise. Again, some of the previous works reviewed show that different parts of the plants (leaves, seed oil, rhizomes) possess antibacterial activity, suggesting that the bioactive principles may we widely distributed in the plant.

Furthermore, the broad specificity of the extract in our study, and other related works, evidenced by its ability to inhibit the growth of gram-positive, -negative bacterial species and fungal pathogens suggest that there may several bioactive principles working in synergy to produce the observed therapeutic effect.

Unfortunately, most of the available literature on the antimicrobial properties of *A. melegueta* are often limited to crude extract without molecular characterization of the bioactive principle, and a mechanistic study into their mode of action. Only a handful of work have ventured into these areas. In the future, effort on isolating and understanding the mechanism of action of the bioactive principles will be very important if the antimicrobial potential of the plant is to be fully realized.

COMPETING INTERESTS

We declare that there is no competing interest for this work; be it financial, personal or organizational that could inappropriately influence (bias) this work.

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