


Phytochemical profile and antibacterial activity of crude extracts of the pod of *Aframomum angustifolium* (Sonn.)...

Claude Kirimuhuzya

Related papers

[Download a PDF Pack](#) of the best related papers 



[Triterpenoids from *Vernonia auriculifera* Hiern exhibit antimicrobial activity](#)

Hafizah Chenia, Neil Koorbanally

[Antibacterial Activity of Naturally Occurring Compounds from Selected Plants](#)

Ivana Radojevic

[Antibacterial and anti-adhesion activity of the pentacyclic triterpenoids isolated from the leaves and ...](#)

Roshila Moodley, Hafizah Chenia, Sreekantha Babu Jonnalagadda

Phytochemical profile and antibacterial activity of crude extracts of the pod of *Aframomum angustifolium* (Sonn.) K. Schum.

Godwin Upoki Anywar * and [Claude Kirimuhuzya](#)

Department of Biological Sciences, College of Natural Sciences, Makerere University, Kampala, Uganda

* Corresponding author: [Anywar Godwin](#), Department of Biological Sciences, College of Natural Sciences, Makerere University, P.O. Box 7062 Kampala, Uganda, Tel: +256 702-983410, Fax: +256 414 531061, e-mail: godwinanywar@gmail.com, ganywar@cns.mak.ac.ug

TMKARPIŃSKI
PUBLISHER



Received: 13 March 2015; Revised submission: 16 June 2015; Accepted: 18 June 2015

Copyright: © The Author(s) 2015. European Journal of Biological Research © Tomasz M. Karpiński 2015. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
www.journals.tmkarpinski.com/index.php/ejbr

ABSTRACT

Aframomum angustifolium (Sonn.) K. Schum., is a perennial herb indigenous to Uganda and is widely used for medicinal and ethnodietary purposes. The ether and methanol extracts of the ripe pod of *A. angustifolium* were screened for antibacterial activity against; *Staphylococcus aureus* (ATCC 43300), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and clinical isolates of *Escherichia coli*. The plant extracts were also analysed for their phytochemical constituents. The Agar-well diffusion assay was carried out to measure the antibacterial activity. The Minimal Inhibitory Concentration (MIC) of the extracts was determined using serial dilution method. The phytochemical analysis of the extracts was done using standard procedures. The MIC of the extracts was 125 mg/ml. The methanol extract showed the strongest antibacterial activity against *S. aureus* (ATCC 25923) of 18.5 mm. Both the methanol and ether extracts tested positive for various phytochemicals especially flavonoids and terpenoids which are known to have antibacterial activity. The

ether and methanol extracts of the ripe pod of *Aframomum angustifolium* possess antibacterial activity, justifying the use of the fruit in ethno-medicine.

Keywords: *Aframomum angustifolium*, Minimal Inhibitory Concentration, Agar-well, Phytochemical, Antibacterial, Ethnomedicine.

1. INTRODUCTION

Aframomum angustifolium is a perennial herb indigenous to Uganda [1]. The genus consists of about 50 species, with about 13 species reported to occur in Uganda [2, 3]. The ripe fruits appear above the soil surface and are often eaten as a snack [1]. *Aframomum* fruits are usually collected from the wild and often sold in markets in Uganda. However, draining of swamps and cutting of riverine forests are threatening the survival of the plant [1].

Traditionally, *Aframomum* species have been used as laxative, antiseptic, antipyretic, analgesic, antischistosomal, carminative and a sexual stimulant. They have also been used to treat dysentery,

snakebites, cataracts, abscesses, oedema, colds, migraine and toothache among others [4-6].

Since prehistoric times, humans have used indigenous plants to treat infectious diseases [7, 8]. In spite of the long history, it was not until the discovery of penicillin that large-scale screening of higher plants for antibacterial substances began [7, 9]. It was reported that less than 5,000 plant species had been studied in depth by 2000 despite the fact that higher plants contributed about 25% of the total of all drugs in clinical use [10, 11].

Many traditional medicinal plants contain compounds with antibacterial activity that often make excellent lead compounds used in drug development [11, 12]. New antibiotics continue to be isolated from nature but most of the time; they are variants of known substances without sufficient advantage to favour their use in place of well-established compounds [13]. Synthetic compounds have also been difficult to find [13, 14]. Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. It is now a growing global public health concern and continues to threaten the successful treatment of infectious disease [12, 15]. It is also now common to hear about methicillin-resistant *Staphylococcus aureus* (MRSA) [12, 14].

Previous studies on the seeds of *Aframomum* have indicated that they have a strong antibacterial activity as well as different active principles [6]. More recently, Ngwoke et al. [12] showed that two of the compounds isolated and purified from the rhizomes of *A. melegueta* G3 & G5b were more potent than Vancomycin, a drug of last resort used in the treatment of MRSA.

Although several authors including [2, 12, 16-19], have carried out research on the different species of *Aframomum*, especially the seeds of the West African *A. melegueta*, no studies have been conducted on *Aframomum angustifolium*. Many of the studies have concluded that although *Aframomum* is a promising therapeutic antimicrobial agent, the high toxicity of the seeds due to the presence of oxalic acid limits its wide use [5, 6, 16, 17, 19]. However, no research has been carried out on *A. angustifolium*. The appropriate utilisation of local resources to cover drug needs is dependent on preliminary scientific studies to determine their

efficacy and safety [20]. This study was therefore carried out to investigate the antibacterial activity of the pod of *A. angustifolium*.

2. MATERIALS AND METHODS

2.1. Study site

The study was carried out in Microbiology laboratory, Department of Medical Microbiology and the Phytochemistry laboratory in the Department of Pharmacology and Therapeutics, College of Health Sciences in Makerere University.

2.2. Collection of the medicinal plant

The plant material was harvested from the wild in Mpigi district in Central Uganda. Voucher specimens of the plant were collected according to standard procedures described in Martin [21] and deposited in the Makerere University Herbarium for identification.

2.3. Extraction of compounds from plant material

Sequential extraction of the plant material with petroleum ether and methanol was carried out. The dried powdered plant material (250 g) was soaked in 500 ml of petroleum ether for 4 days. The mixture was decanted and filtered using Whatman filter paper and the residue was air-dried for 2 days. After drying, the same plant material was soaked in methanol for four days and the same procedure was repeated.

2.4. Phytochemical screening

Phytochemical screening of crude plant extracts was conducted following the procedures described in Sofowora [22], and Trease and Evans [6].

2.5. Preparation of plant extracts

The fruit pod was stripped off and air-dried at room temperature for two weeks. The dried material was then ground into a fine powder using a mortar and pestle to facilitate the extraction process. The dry plant extracts were obtained by recovering the solvent used for dissolving the powder using a

rotary evaporator (Büchi® rotary evaporator Model R-205). A stock solution of 250 mg/ml was prepared for the bioassays by weighing 2.5 g of the extract and dissolving it in two drops of dimethylsulphoxide (DMSO). The stock solution was kept at 4 °C and varying concentrations were made from it for the bioassay.

2.6. Bacterial selection criteria

S. aureus (ATCC 25923) and *S. aureus*, (ATCC 43300) *E. coli* (ATCC 25922) and clinical isolates of *E. coli* were used since they represent typical Gram-positive and Gram-negative organisms respectively, and are the two most common organisms used by other researchers [9, 23]. They are also implicated in food poisoning, urinary tract infections, blood stream infections and wound infections [15, 24]. *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) are known to be sensitive to antibiotics. *S. aureus* (ATCC 43300, MRSA) and clinical isolates *E. coli* that were known to be resistant were selected. It was in the interest of the study to use both known resistant and susceptible strains of the test organisms, for comparison purposes. Clinical isolates of *E. coli* which has shown resistance to different antibiotics were used. The bacteria were obtained from the freezer and incubated for 18 hours. The isolates were subcultured to obtain pure isolated colonies, which were used for the assays.

2.7. Preparation of the medium

The medium was prepared by adding 40 g of Mueller-Hinton agar powder (Sigma-Aldrich Inc.) to one liter of distilled water and then boiling the mixture. The solution was autoclaved at 121 °C at 15 psi for 15 minutes and cooled to 50 °C in a water bath. It was then transferred into sterile Petri dishes. It was allowed to cool and solidify under sterile conditions, and then incubated for 24 hours at 37 °C to ensure that there is no microbial contamination.

2.8. Agar-well diffusion assay

The Mueller-Hinton agar plates were inoculated in three planes using sterile cotton tipped

swabs. Cultures of *S. aureus* (ATCC 2592), MRSA (ATCC 43300), *E. coli* (ATCC 25922), and the clinical isolates of *E. coli* were inoculated separately on the solidified agar on each Petri dish. About 250 mg/ml of the test extracts were used. Oxacillin was used as the positive control, while Dimethyl Sulphoxide (DMSO) and a blank well were used as negative controls. The extracts and controls were dispensed into the uniformly cut wells of 10 mm diameter, which were filled to about $\frac{3}{4}$ of their height. The plates were incubated at 37 °C for 24 hours without inverting them. The sensitivity of the test organisms to the extracts was determined by measuring the diameters of the zone of inhibition surrounding the wells with a metric ruler. A zone devoid of growth around the well indicated the capacity of the plant extract to inhibit growth.

2.9. Determination of Minimal Inhibitory Concentration (MIC) by serial dilution method.

The MIC values were determined by preparing two dilutions of the stock extract solution in standard nutrient broth (Sigma-Aldrich Inc). Two test tubes were arranged in a row and serial dilutions of the crude extracts were carried out with 250 mg/ml as the highest concentration in tube 1. About 0.5 ml of distilled water was poured in each test tube, and then 0.5 ml from tube 1 was poured in tube 2. It was mixed well and the process repeated to produce two dilutions. Turbidity was used as a growth indicator and growth was compared with a 0.5 McFarland inoculum.

3. RESULTS AND DISCUSSION

3.1. Qualitative phytochemical tests

Phytochemical analyses were conducted on twelve phytochemical compounds in both the ether and methanol extracts (Table 1). Triterpenoids, carotenoids, carbohydrates, flavanoids and coumarins were found in both the ether and methanol extracts. Tannins, basic alkaloids and reducing sugars were found only in the methanol extract whereas triterpenoids/steroids, unsaturated compounds and pentose sugars were limited to the ether extract.

Table 1. Phytochemical analysis of the crude extracts of the fruit pulp of *A. angustifolium*.

Compound	Methanol extract	Ether extract
1. Triterpenoids/steroids	+	++
2. Tannins	+	-
3. Basic alkaloids	+	-
4. Coumarins	+	+
5. Flavanoids	++	+
6. Saponins	-	-
7. Anthraquinones	-	-
8. Reducing sugars	+	-
9. Pentose sugars	-	+
10. Carbohydrates	+	+
11. Carotenoids	++	+
12. Unsaturated compounds	-	+

Key: ++ = Strongly present, + = Present, - = Absent

3.2. Agar-well diffusion assay

The diameters of inhibition of both the methanol and ethanol extracts show that the plant has antibacterial activity (Table 2). Both the ether extracts (14.8 mm) and methanol extracts (16.2 mm) had greater diameters of inhibition than the positive control (11.7 mm) against *S. aureus* (ATCC 43300). The ether extract showed the strongest antibacterial activity against *S. aureus* (ATCC 25922) with diameter of zone of inhibition of 18.5 mm. The methanol extract had the strongest antibacterial activity against *S. aureus* (ATCC 43300) (16.2 mm).

Diameters of the zones of inhibition include the diameters of the wells (10 mm). Both strains of *E. coli* had similar inhibition zones to the positive control. *E. coli* strains were also the most resistant strains to the extracts. Generally, the methanol extract produced larger diameters of inhibition than the ether extract. The lowest concentration that inhibited growth of the bacteria (Minimal Inhibitory Concentration) was found to be 125 mg/ml of plant extract.

Table 2. Diameters (mm) of the zones of inhibition of the extracts.

Plant Extracts	<i>S. aureus</i> (ATCC 25923)	<i>S. aureus</i> (ATCC 43300)	<i>E. coli</i> (ATCC 25922)	<i>E. coli</i> (Clinical isolates)
Ether	18.5±0.50	14.8±0.29	10.8±0.29	12.0±0.80
Methanol	14.5±0.50	16.2±0.29	12.2±0.76	12.0±0.50
Positive control	44.5±0.29	11.7±0.29	12.3±0.58	13.0±0.00

Values are means ± standard deviation (SD).

4. DISCUSSION

Although other researchers have largely studied the seeds of different *Aframomum* species, a comparison shows similar phytochemical composition and biological activity with the pod extracts of *A. angustifolium*. For example, Gröblacher [18] reported the presence of saponin, tannins, alkaloids, steroid, cardioglycosides, flavonoids, and terpenoids in the seed extract of *Aframomum melegueta*. Similar phytochemicals have been detected in the pod extracts of *A. angustifolium* from this study.

Aframomum species are best known for the production of labdane diterpenoids and flavonoids [2, 12] isolated, purified and tested the labdane

diterpenes G3 and G5 from the rhizomes of *A. melegueta* against *E. coli*, *L. monocytogenes* and MRSA. These compounds were shown to exhibit more potent antibacterial activity compared to the current clinically used antibiotics ampicillin, gentamicin and vancomycin and can be potential antibacterial lead compounds. Similarly, this study showed both the ether and methanol extracts of *A. angustifolium* to possess greater antibacterial activity against *S. aureus* (ATCC 43300) than the positive control Oxacillin. The greater antibacterial activity of the plant extracts against the Gram-positive *S. aureus* strains is in agreement with what is known about antibacterial diterpenoids. According to Porto et al. [25] and Fonseca et al. [26] antibacterial diterpenoids are known to have

potent antimicrobial activity against Gram-positive organisms.

Cousins and Huffman [27] found the seeds of *A. giganteum* to be rich in flavonoids, particularly quercetin and kaempferol both of which possess antibacterial and antifungal activities. Ayafor, et al. [16] also found the seed extracts of *A. danielli* and *A. aulacocarpos* to contain Aframodial [1] and other bioactive diterpenoids. It is interesting to note that both the ether and methanol pod extracts of *A. angustifolium* were rich in flavonoids. The ether extracts of the pod of *A. angustifolium* from this study also tested positive for basic alkaloids. Tane et al. [2] also reported the presence of hydroxyphenyl alkaloids in the seed extracts of *A. melegueta*.

The antibacterial activity exhibited by the pods of *A. angustifolium* is in agreement with the findings of Ayafor, et al. [16] and Cousins and Huffman, [27] who reported diterpenoids from *A. aulacocarpos*, Labdane diterpenoids from *A. albo-violaceum* and flavonoids from *A. giganteum* to possess antifungal, antiviral and other bioactivity. Although the essential oil composition of the pod of *A. angustifolium* was not examined in this study, we can conjecture that it is highly likely to also contain essential oils. This can be supported by research by Eyob et al. [28] who demonstrated that the pods of *A. corrorima* had different types of essential oil components, the major one being γ -terpinene (27.1%) with a typical odour.

CONCLUSION

The extracts of the pod of *A. angustifolium* have antibacterial activity against *S. aureus* ATCC 25922, *S. aureus* ATCC 43300, clinical isolates of *E. coli* and *E. coli* ATCC 25922 *in vitro*. They also contain phytochemical compounds, some of which have proven antibacterial properties.

AUTHORS' CONTRIBUTION

GUA wrote the original concept and study design and participated in drafting the original manuscript and carrying out the phytochemical analyses. Both GUA & CK participated in carrying out the antibacterial laboratory analyses. Both GUA & CK participated in writing subsequent drafts on the

manuscript, and have both read and approved the final version.

ACKNOWLEDGEMENTS

We like to thank Dr. A. L. Upoki for the financial support to conduct this study. We also extend our gratitude to Prof. Olwa-Odyek (RIP), Mrs. W. Atuhaire, Mr. H. Baluku and Dr. F. Bwanga for granting us permission to use their laboratories and Assoc. Prof. M.-M. Kamatenesi for technical guidance.

TRANSPARENCY DECLARATION

The authors declare that they have no competing interests.

REFERENCES

1. Katende AB, Segawa P, Birnie A, Tegnäs B. Wild food plants and mushrooms of Uganda. Sida RELMA Technical Handbook No. 19 Nairobi; 1999.
2. Tane P, Simplicite DT, Ayimele GA, Connolly JD. Bioactive metabolites from *Aframomum* species. Proceedings of the 11th NAPRECA Symposium 9-12 August 2005: 214-223.
3. Lye LK, Bukenya-Ziraba R, Waako P, Tabuti JRS. Plant-medicinal dictionary for East Africa. Makerere University Herbarium Department of Botany Kampala Uganda, 2008.
4. Kokwaro J. Medicinal plants of East Africa. 2nd edn. Nairobi Kenya Literature Bureau, 1993.
5. Duke AJ, Bogenschutz-Godwin JM, duCellier J, Duke PAK. Handbook of medicinal herbs. 2nd edn. CRC Press London, 2006.
6. Trease ECW, Evans WC. Pharmacognosy. 15th edn. Elsevier Ltd. London, 2004.
7. Ray PG, Majumdar SK. Antimicrobial activity of some Indian plants. Econ Bot. 1977; 30 (4): 317-320.
8. Nostro A, Germanó MP, D'Angelo VD, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol. 2000; 30: 379-384.
9. Rola CFJr, Smith R. Antibacterial screening of some ornamental plants. Econ Bot. 1977; 31(1): 28-37.

10. Swerdlow JL. *Natures medicines plants that heal*. National Geographic Society Washington D.C, 2000.
11. Van Wyk BE, Wink M. *Medicinal plants of the world*. Timber Press Inc. Oregon, 2004.
12. Ngwoke KG, Chevallier O, Wirkom VK, Stevenson P, Elliott CT, Situ C. *In vitro* bactericidal activity of diterpenoids isolated from *Aframomum melegueta* K.Schum against strains of *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus*. *J Ethnopharmacol*. 2014; 151(3): 1147-1154.
13. Franklin TJ, Snow GA. *Biochemistry of antimicrobial action*. Chapman and Hall London, 1985.
14. Russell AD. Antibiotic and biocide resistance in bacteria. *J Appl Microbiol*. 2002; 92 Suppl: 1S-3S.
15. WHO. *Antimicrobial resistance: global report on surveillance*. World Health Organization, 2014.
16. Ayafor JF, Tchuendem MHK, Nyasse B, Tillequin F, Anke H. Aframodial and other bioactive diterpenoids from *Aframomum* species. *Pure Appl Chem*. 1994; 66(10/11): 2327-2330.
17. Ilic N, Schmidt BM, Pouleva A, Raskina, I. Evaluation of grains of paradise (*Aframomum melegueta*) [Roscoe] K. Schum. *J Ethnopharmacol*. 2010; 127 (2): 352-356.
18. Gröblacher B, Maier V, Kunert O, Bucar F. Putative mycobacterial efflux inhibitors from *Aframomum melegueta*. *J Nat Prod*. 2012; 75: 1393-1399.
19. Akpanabiatu MI, Ekpo ND, Ufot UF, Udoh NM, Akpan JE, Etuk EU. Acute toxicity, biochemical and haematological study of *Aframomum melegueta* seed oil in male Wistar albino rats. *J Ethnopharmacol*. 2013; 150(2): 590-594.
20. Bannerman HR, Burton J, Wen-Chieh C. *Traditional medicine and health care coverage*. WHO Macmillan/Spottiswoode, 1983.
21. Martin GJ. *Ethnobotany: a methods manual*. Chapman & Hall, London, 1995.
22. Sofowora A. *Medicinal plants and traditional medicine in Africa*. Spectrum Books, Ibadan, 1993.
23. Heritage J, Evans EGV, Killington RA. *Introductory Microbiology*. Cambridge University Press, 1996.
24. Eley RA. *Microbial food poisoning*. Chapman and Hall London, 1992.
25. Porto TS, Simão MR, Carlos LZ, Martins CH, Furtado NA, Said S et al. Pimarane-type diterpenes obtained by biotransformation: antimicrobial properties against clinically isolated Gram-positive multidrug-resistant bacteria. *Phytother Res*. 2013; 27(10): 1502-1507.
26. Fonseca AP, Estrela FT, Moraes TS, Carneiro LJ, Bastos JK, Santos RA et al. *In vitro* antimicrobial activity of plant-derived diterpenes against bovine mastitis bacteria. *Molecules*. 2013; 18(7): 7865-7872.
27. Cousins D, Huffman MA. *African study monographs*. 2002; 23: 65.
28. Eyob S, Appलगren M, Rohloff J, Tsegaye A, Messele G. Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (*Aframomum corrorima* (Braun) P.C.M. Jansen) from southern Ethiopia. *South Afr J Bot*. 2008; 74(2): 181-185.