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Antiviral activity of aframomum melegueta against severe acute respiratory syndrome coronaviruses type 1 and 2

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1. Introduction

As of June 2021, the COVID-19 pandemic was a worldwide health crisis. Several variants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have emerged, increasing the virus's virulence potential by making it more contagious (Winger and Caspari, 2021). Thus, there is a need to identify antiviral compounds that could treat or prevent SARS-CoV-2 infections. Phytotherapy constitutes a vast source for identifying such compounds.

The African spice Aframomum melegueta K. Schum. is a member of the ginger family (Zingiberaceae). It is native to Africa, where its seeds are known as grains of paradise. Omotuyi et al. (2021) suggested that A. melegueta seed components might have antiviral activity against SARS-CoV-2. They computationally screened 100 compounds previously characterized from A. melegueta for antiviral activity against known SARS-CoV-2 targets. Their models indicated that several seed compounds had the potential to inhibit virus attachment and entry to cells (Omotuyi et al., 2021). Although not explored by Omotuyi et al., gingerols, the main non-volatile components in A. melegueta, might contribute to antiviral activity against SARS-CoV-2 (Juliani et al., 2008).

This study sought to determine the antiviral activity and phytochemical composition of six A. melegueta commercial seed products. In vitro antiviral activity of crude ethanolic extracts and selected individual components was determined using the XTT cytotoxicity assay.
Aframomum melegueta extracts, 6-gingerol, and tectochrysin show moderate antiviral activity against SARS-CoV-1 and 2 PsVs.

| Commercial product samples          | Seed extract (SE) # | EC50 (mg/mL) | TI | EC50 (mg/mL) | TI | EC50 (mg/mL) | TI | EC50 (mg/mL) | TI | EC50 (mg/mL) | TI |
|-------------------------------------|---------------------|--------------|----|--------------|----|--------------|----|--------------|----|--------------|----|
| Grains of Paradise, Fronair Co-op, Norway, IA | 1                   | 3.94         | 0.59 | 6.7          | 0.45 | 8.7          | 0.72 | 5.5          | 0.59 | 6.7          | 0.72 |
| Grains of Paradise, Eder Gewurze, Mattighofen, Austria | 2                   | 2.92         | 0.54 | 5.4          | 0.41 | 7.1          | 0.55 | 5.3          | 0.53 | 5.5          | 0.53 |
| Grains of Paradise, Spice Specialist, Hicksville, NY | 3                   | 2.22         | 0.35 | 4.0          | 0.49 | 4.7          | 0.36 | 3.1          | 0.26 | 3.1          | 0.26 |
| Grains of Paradise, The Spice Lab, Fort Lauderdale, FL | 4                   | 3.08         | 0.39 | 5.1          | 0.49 | 4.9          | 0.36 | 2.2          | 0.26 | 2.2          | 0.26 |
| Grains of Paradise, North Mountain, Muncy Valley, PA | 5                   | 3.08         | 0.57 | 5.4          | 0.33 | 9.4          | 0.49 | 6.3          | 0.75 | 4.1          | 0.75 |
| Grains of Paradise, Dee Online Store Inc., Miami, FL | 6                   | 3.36         | 0.28 | 11.8         | 0.26 | 13.1         | 0.27 | 12.6         | 0.55 | 12.6         | 0.55 |

Ethanol extracts and powdered material were tested against SARS-CoV-1 and 2 PsVs. EC50 values were calculated from those values. Antiviral activity was tested using the SARS-CoV-1 pseudovirus (PsV) and several variant strains of SARS-CoV-2 PsV. SARS-CoV-2 PsV strains tested in this study included pseudoviral particles that display the original Wuhan strain spike and spikes with mutations in the receptor-binding domain recently identified in the United Kingdom (501Y.V2), South Africa (B.1.351), and Brazil (P1) (Schmidt et al., 2020).

2. Materials and methods

2.1. Plant materials, ethanol extraction and potential active compounds

A. melegueta seeds from six commercial sources and potential active compounds were tested (Table 1). Seeds were ground for 30 s using a coffee grinder (Mr. Coffee, Cleveland, Ohio). For each sample, six mg of powdered material were mixed with 3 mL of 95% ethanol (Sigma Aldrich, St Louis, MO) using a vortex mixer. Samples were then sonicated three times at 10-second intervals and 30% intensity using a Bransonic® Ultrasonic bath (Branson Ultrasonics, Brookfield, CT). Samples were spun in an Eppendorf 5804R centrifuge at 300 g for 5 min, and the supernatant was collected and filtered through a 0.45 μm cellulose acetate syringe filter (Thermo Fisher Scientific, Waltham, MA). Extracts were aliquoted and stored at –20 °C.

2.2. Cytotoxicity and antiviral assay

HeLa-ACE2 cells were used to estimate cytotoxicity (toxic to living cells) and antiviral activity (capability to inhibit pseudovirus entry). The HeLa-ACE2 cells are human cervical adenocarcinoma cells engineered to express the human angiotensin-converting enzyme 2 (hACE-2) receptor on its surface. Both SARS-CoV-1 and SARS-CoV-2 bind to the hACE2 receptor, expressed on HeLa-ACE2 cells for attachment and entry to start the viral replication cycle (Zamorano Cuervo and Grandvaux, 2020). The pseudoviral particles can be used in this cell-based system to mimic virus attachment and entry. This process can be measured by the PsV ability to deliver the reporter genome into these cells, resulting in luciferase expression and luminescence[7].

XTT and cell-based pseudoviral assays were performed to test cytotoxicity and antiviral activity, respectively, as previously described in (Melo et al., 2021). For the cytotoxicity assay, HeLa-ACE2 cells were exposed to different seed extract dilutions, compound dilutions, or culture medium (Dulbecco Modified Eagle Medium containing 10% fetal bovine serum) containing antibiotics (Thermo Fisher Scientific) to serve as cell controls. After 72 h of incubation at 37 °C, 5% CO2, and 98% humidity, cell viability was estimated using the XTT colorimetric assay. The 96-well microplates were read at 450 nm using the Spectromax iD3 microplate reader (Molecular Devices, San Jose, CA).

To test for antiviral activity, the different extract dilutions tested in the cytotoxicity assay or cell culture medium (for virus control) were preincubated with SARS-CoV-1 PsV, SARS-CoV-2 Wuhan PsV, SARS-CoV-2 D614G PsV or SARS-CoV-2 K1417N/E484K/N501Y PsV (Schmidt et al., 2020) for 30 min at 37 °C, 5% CO2, and 98% humidity. After incubation, samples were transferred to 96-well white-opaque microplates (Thermo Fisher Scientific) containing HeLa-ACE2 cells and incubated for 72 h at 37 °C, 5% CO2, and 98% humidity. Luciferase activity was tested using the TurboLuc One Step assay (Thermo Fisher Scientific) (Melo et al., 2021). Luminescence was read in the Spectromax iD3 microplate reader.

Raw data from the XTT and cell-based pseudoviral assays were analyzed using GraphPad Prism software version 9.0.2 (San Diego, CA) to obtain the half-maximal cytotoxic concentration (CC50) and the half-maximal effective concentration (EC50). All extract dilutions and controls were tested in triplicate in two independent
experiments. CC50 and EC50 ratios were used to calculate the therapeutic index (TI).

2.3. Combination studies

The potential additive, synergistic or antagonistic effect was estimated using CalcuSyn software (Biosoft, Cambridge). The software estimates the combination index (CI) values and is an analyzer of combined drug effects (He et al., 2018). The percentage of pseudoviral entry inhibition described in Section 2.2 was used to analyze the effect of the combination of 6-gingerol and tectochrysin. For this purpose, equipotential concentrations (between 1.9 and 500 μM) of 6-gingerol and tectochrysin were tested alone and in combination.

2.4. Chromatographic analysis

Analysis was performed on an Agilent 1290 UPLC/DAD in tandem with an Agilent 6546 QTOF/MS with U.V. spectra wavelengths of 254, 280, and 350 nm. Chromatographic conditions: EclipsePlus C18 (1.8 um, 2.1 × 50 mm) column, gradient of water + 0.1% formic acid (solvent A) and acetonitrile + 0.1% formic acid (solvent B), column at 30 °C, and flow rate of 0.3 ml/min. Gradient began at 20% B and was raised to 30% B over 5 min, after which gradient B was changed from 30 to 80% over the next 25 min, followed by B isocratic elution for 3 min and 20% B for 1.5 min for column equilibration. MS parameters: gas temperature 300 °C, dry gas flow rate 11 L/min, nebulizer pressure 25 psi, sheath gas temperature 350 °C, and 11 L/min flow rate. The capillary voltage set to 3500 V, the nozzle voltage to 1000 V, and the fragmentor to 100 V. Positive ion mode was utilized along with an ESI source. MassHunter Qualitative Analysis was used for data analysis. METLIN (and a personal PCDL were scored for gingerols an ESI source. MassHunter Qualitative Analysis was used for data

Fig. 1. Aframomum melegueta seeds and the chemical structure of potential active compounds. A) 6-gingerol, identified in this study; B) tectochrysin, identified by Omotuyi and collaborators (Omotuyi et al., 2021).
Table 2

Gingerols and other non-volatile compounds in A. melegueta ethanolic seed extracts.

| Seed extract #* | 1 | 2 | 3 | 4 | 5 | 6 |
|----------------|---|---|---|---|---|---|
| Mean ± SE (mg / 100 g dry weight) |   |   |   |   |   |   |
| 4-Gingerol | 0.41±0.03 | 0.27±0.01 | 0.26±0.02 | 0.24±0.02 | 0.21±0.01 | 0.59±0.04 |
| 8-dehydrogingerdione | 4.27±0.31 | 2.97±0.16 | 2.88±0.12 | 2.69±0.09 | 2.59±0.05 | 4.00±0.08 |
| Acetoxy-6-gingerol (dihydroxyphenyl) : (dihydroxyphenyl) heptane | 16.13±0.65 | 15.68±0.33 | 15.07±0.21 | 12.06±0.19 | 13.34±0.30 | 16.54±0.37 |
| 6-Gingerol | 1.56±0.13 | 2.18±0.12 | 2.03±0.09 | 1.97±0.06 | 1.83±0.08 | 0.91±0.02 |
| 6-Gingerol | 127.73±5.21 | 92.65±3.03 | 87.72±2.45 | 61.57±1.51 | 74.00±1.69 | 147.97±4.04 |
| dehydro-hydroxygingerol | 11.74±0.62 | 10.60±0.36 | 9.84±0.09 | 8.31±0.30 | 8.78±0.40 | 13.53±0.38 |
| Methylgingerol | 13.17±0.74 | 9.03±0.45 | 8.88±0.25 | 5.00±0.36 | 7.82±0.28 | 10.56±0.61 |
| Acetoxy-6-gingerol | 4.40±0.24 | 2.48±0.11 | 2.07±0.03 | 1.95±0.08 | 2.05±0.05 | 5.63±0.26 |
| 6-Gingerol | 4.90±0.18 | 3.97±0.11 | 4.37±0.10 | 3.38±0.04 | 3.14±0.07 | 5.44±0.12 |
| 6-Shogaol | 35.54±1.74 | 26.92±0.63 | 15.71±0.54 | 25.53±0.56 | 26.19±0.57 | 15.32±0.32 |
| dehydro-hydroxygingerol 2 | 12.29±0.62 | 11.10±0.43 | 10.15±0.07 | 8.25±0.20 | 8.74±0.28 | 15.94±0.48 |
| 6-Paradol | 10.88±0.23 | 11.48±0.34 | 9.53±0.20 | 9.51±0.17 | 9.58±0.27 | 10.10±0.37 |
| 1-Dehydro-6-gingerdione | 4.42±0.32 | 3.15±0.10 | 1.60±0.09 | 1.99±0.08 | 3.01±0.11 | 1.21±0.04 |
| (α,β)-Gingerol | 0.22±0.00 | 0.18±0.02 | 0.21±0.01 | 0.15±0.01 | 0.15±0.01 | 0.19±0.01 |
| Methyl 10-gingerol | 0.25±0.01 | 0.20±0.02 | 0.27±0.01 | 0.13±0.00 | 0.12±0.00 | 0.52±0.02 |
| Total Gingerols | 175.11 | 130.48 | 134.82 | 88.99 | 105.02 | 200.36 |

Table 3

Calcinesin summary table for 6-gingerol and tectochrysin combination.

| Active Compound | CI values at Dm r |
|----------------|------------------|
| 6-gingerol | N/A | N/A | N/A | 102.6 | 0.92983 |
| Tectochrysin | N/A | N/A | N/A | 64.9 | 0.83323 |
| 6-gingerol + tectochrysin | 0.87148 | 0.87482 | 0.94258 | 34.6 | 0.98454 |

N/A: Not Applicable; CI: is the combination index and is a quantitative measure of the degree of drug interaction; CI around 1, additive effect; CI < 0.8, synergistic effect; CI > 1.3, antagonism; Dm is the median-effect dose; r is the linear correlation coefficient of the median-effect plot.

### 4. Conclusions

In conclusion, a promising anti-SARS-CoV-1 and 2 activity of A. melegueta seed extracts was observed and seemed to be due to the presence of 6-gingerol and other identified components. Combined 6-gingerol and tectochrysin showed additive antiviral activity against SARS-CoV-2, suggesting they are active components in A. melegueta’s seed extracts.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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