Potential antioxidant, cytotoxic, antiviral, and antibacterial activities of extracts from three species of *Piper*

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BACKGROUND

Natural plant extracts have a long history of being used in traditional medicine and western pharmaceuticals to treat ailments such as respiratory disorders, infections, inflammatory disorders, and pain[1,2]. The genus *Piper*, of the family *Piperaceae*, contains many species with medicinal properties, examples of which include *Piper nigrum, Piper guineense,* and *Piper borbonense*. In many countries around the world, peppers of the genus *Piper* are commonly used as spices and traditional medicines.

The aim of this study was to identify total phenolic content and antioxidant, cytotoxic, antiviral, and antibacterial activities of extracts from seven commercial sources of *P. nigrum, P. guineense,* and *P. borbonense.*

MATERIALS AND METHODS

Plant materials: Seven commercial samples of dried *Piper* peppercorns were attained from the following sources: *Piper nigrum* (McCormick), *Piper guineense* (from Nigeria), and *Piper borbonense* (from Madagascar) (Table 1).

Extraction Procedure: Samples were ground using a coffee grinder. Ground peppercorns were suspended in sterile distilled water or ethanol to a concentration of 20 to 40 mg/mL. Suspensions were sonicated in a Cole-Parmer 08895-04 sonicator at 100%, three times for two-minute intervals. Following centrifugation, extracts were further filtered with a 0.45 µm non-polar syringe filter and stored at 4°C. Extracts used for Figure 3 were further filtered through a 0.2 µm non-polar syringe filter.

Total phenols: Phenols were quantified using a modified Folin-Ciocalteu's protocol[3] measuring absorbance at 765 nm. Total phenolic content (TPC) was expressed as mg gallic acid equivalents/mL plant extract (GAE mg/mL).

Antioxidant capacity: The ABTS method was used to determine the anti-oxidant activity of the commercial samples measuring the absorbance at 734nm[4]. Results are expressed as mg Trolox equivalent/ml plant extract (TEAC mg/ml).

Cytotoxicity and Antiviral Activity: The cytotoxicity in the Caco-2 epithelial cell line and viral entry inhibition by aqueous extracts were explored using the XTT colorimetric assay and the SARS-CoV-2 Delta variant pseudoviral model as described by Melo *et al.,* 2021[5].

Antibacterial activity: Fresh overnight cultures of both *E. coli* and *B. subtilis* were prepared to use in these experiments. For each well of a 96-well microplate, 300 µL of LB broth +/- 20 mg/mL *Piper* extract was inoculated with 10^5 cells/mL of either fresh *E. coli* or *B. subtilis* and grown in. Microplates were incubated at 37°C and 150 rpm. Growth of bacteria was recorded at one-hour intervals by measuring optical density at 600 nm in a Biotek GenSys 5 microplate reader. Two readings were recorded for each culture with 10 seconds of agitation before each reading.

Table 1. *Piper* samples, country of origin and commercial source.

Sample	Country	Commercial Source	Code
Piper nigrum	India	McCormick and Co., Inc.	PN1
Piper guineense	Nigeria	Worldfood Store	PG1
Piper guineense	Nigeria	Darmol African Market	PG2
Piper guineense	Nigeria	Foodsby Testimony	PG3
Piper borbonense	Madagascar	Sama Market	PB1
Piper borbonense	Madagascar	"Pili Pili Dack" Madepices	PB2
Piper borbonense	Madagascar	Floribis	PB3

RESULTS

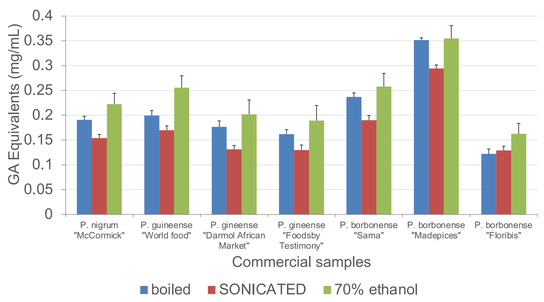

Figure 1. Total phenolic content (TPC, mg gallic acid equivalents/mL) in commercial samples of African peppers. Bars indicate means ± standard error.

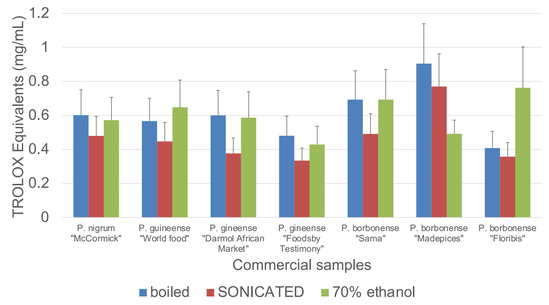

Figure 2. Antioxidant capacity (TEAC, mg Trolox equivalent/mL plant extract) in commercial samples of African peppers. Bars indicate means ± standard error.

Table 2. Aqueous extracts from *P. borbonense have* the best antiviral activity against SARS-CoV-2 delta PsV.

/	PN1	PG1	PG2	PG3	PB1	PB2	PB3
CC_{50}	> 8 mg/mL	1.5 mg/mL	2.9 mg/mL	>8 mg/mL	12.7 mg/mL	10.4 mg/mL	10.7 mg/mL
95% CI	N.A.	0.7 to 3.4	1.7 to 5.4	N.A.	10.6 to 15.4	9.1 to 11.9	8.7 to 13.4
EC_{50}	> 8 mg/mL	3.1 mg/mL	1.9 mg/mL	3.7 mg/mL	1.4 mg/mL	0.7 mg/mL	1.3 mg/mL
95% CI	N.A.	2.2 to 3.7	0.8 to 5.2	2.3 to 5.8	1.1 to 1.8	0.5 to 0.9	1.0 to 1.7
TI Values*	N.A.	0.5	1.5	>2.1	9.1	14.9	8.2

CC_{50}= Half maximal cytotoxic concentration.
EC_{50}= Half maximal effective concentration that inhibits viral entry.
95% CI = 95 % Confidence Interval.
N.A.= Not Applicable
*Therapeutic Index (TI= CC_{50}/EC_{50}). TI values above 10 indicate potential selective antiviral activity.

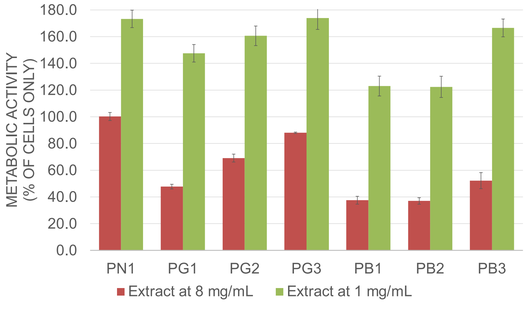

Figure 3. Exposure of Caco-2 cells to the extracts at 8 mg/mL vs. 1 mg/mL suggests a differential impact on the metabolic activity of the cells. Caco-2 intestinal epithelial cells were exposed to either 8 mg/mL or 1 mg/mL of the extracts for 24 hours. Metabolic activity of the cells was assessed after 24 hours using XTT assay. The results are expressed as a percent of control (cells only). PN, *Piper nigrum;* PG, *Piper guineense*; PB, *Piper borbonense.* This was a single trial with three replicates (mean +/- standard error shown) and will be further repeated.

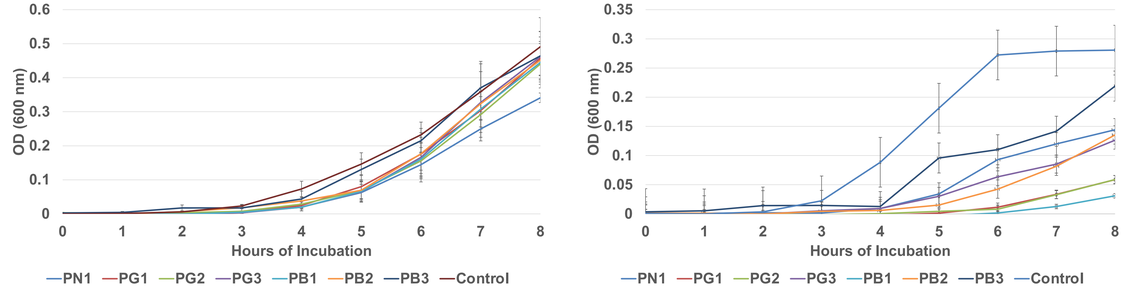

Figure 4. Antibacterial activity. Left, Average growth of *E. coli* in LB +/- *Piper* extract. Right, average growth of *B. subtilis* in LB +/- *Piper* extract. Results shown are the averages of three separate trials of growth from independent cultures. Bars indicate means ± standard error. PN, *Piper nigrum;* PG, *Piper guineense*; PB, *Piper borbonense.*

DISCUSSION AND CONCLUSION

Variations in total phenolic (TP) content and antioxidant capacity between samples and extraction solvents were observed (Fig.1 and 2). Samples with high total phenolics (PB2) exhibited the highest antioxidant capacity. Highest amounts of TP were obtained in boiled extracts, although sonicated extracts were used for the following biological activities.

The SARS-CoV-2 Delta variant pseudoviral model in HeLa ACE-2 cells showed half-maximal effective concentrations (EC_{50} values) between 0.7 and 3.7 mg/mL. The half-maximal cytotoxic concentration and EC_{50} ratio (therapeutic index) showed promising viral entry inhibition in three of seven extracts with selective indexes between 8.2 and 14.9. Aqueous extracts from *P. borbonense* showed the best antiviral selectivity.

The intestinal epithelial cell line, Caco-2, showed a decrease (12-63%) in metabolic activity (XTT) when exposed to the higher concentration of PG and PB extracts (8 mg/ml), suggesting a possible cytotoxic effect. This trend was reversed at the lower concentration (1 mg/ml) of all extracts tested and the cells displayed an increase (23-73%) in activity.

Whereas *P. guineense* and *P. borbonense* extracts inhibited growth of *B. subtilis*, there was no activity observed against *E. coli* (Fig. 4). Differential results are possibly due to differences in bacterial cell wall structure. Samples of *P. borbonense* that exhibited the highest suppression of *B. subtilis* growth had relatively high phenolic content and antiviral activity.

To conclude, the data collectively support a scientific basis for traditional health benefits of *Piper* extracts and warrant further investigation into the actions of specific phenolic compounds present in these extracts and their potential biological activities.

This research provides educational experiences in an urban community college setting that enables students to acquire the critical thinking and research skills necessary to pursue a baccalaureate degree in a science-related discipline.

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