European Online Journal of Natural and Social Sciences 2022;
Vol.11, No 2 pp. 302-308
ISSN 1805-3602

Evaluation of the Antimicrobial Activity of American Cockroach (*Periplaneta americana*) Ethanolic Tissue Extract against Selected Enteric Pathogens

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Received for publication: 15 December 2021.
Accepted for publication: 04 February 2022.

Abstract

Gastrointestinal (GI) tract infections caused by pathogenic microorganisms have a significant role in the global increase in mortality. This issue sparked an investigation into metabolites derived from numerous organisms that may have antimicrobial property against bacterial infections. The Kirby-Bauer Disc Diffusion method was used to test the extract of the American cockroach (*Periplaneta americana*) against enteric bacteria. The results indicate that the ethanolic extract of *P. americana* exhibited antimicrobial activity against the test pathogens, with the greatest inhibitory activity against *Vibrio parahaemolyticus* ($p = 0.00013$) and *Candida albicans* ($p = 0.000911$), when compared to the antibiotic controls Rifampicin, Trimethoprim, Ofloxacin, Penicillin, and antifungal drug Nystatin. However, there was no evidence of inhibitory activity against *Enterococcus faecalis*, *Salmonella enteritidis*, and *Serratia marcescens*. Thus, the current findings indicate that *P. americana* tissue extract may have antibacterial activity against medically important pathogens.

Keywords: Natural product, antimicrobial, *Periplaneta americana*, enteric bacteria, ethanolic tissue extract

Introduction

Infections of the gastrointestinal (GI) tract account for a substantial portion of the burden of disease-related illness globally (Ellis, 2013). Each year, an estimated 1.5 billion incidents of diarrheal complications result in the deaths of around 2.2 million people, the majority of whom are children. The high mortality rate caused by the GI tract infections occur in underdeveloped nations (Mandeville et al., 2009). GI tract infections can range in severity from minor to severe and can impair both the digestive system and general health. Personal hygiene and lifestyle choices are critical for digestive health. Contaminated food consumption may influence digestive upsets, while unsanitary dining preparation or other unsanitary activities may result in the transfer of potentially dangerous microorganisms to foods, resulting in GI infections (Sousa, 2008). According to a local report from the Department of Health (DOH), bloody diarrheal incidents increased 20% from 2016 to 2017. Many of the GI infections are caused by microorganisms, for instance, the enterotoxigenic bacteria can enter the system and produce enterotoxins that affect various parts of the gastrointestinal tract. The alarming resistance of these microorganisms may present a future challenge. Thus, these issues must be addressed through research, as many of these diseases are difficult to detect, their symptoms are poorly controlled, and innovative therapies and treatment source are urgently required (Greenwood-Van et al., 2017).

Current treatments for GI tract infections are symptom-focused and beneficial for just a few subgroups, but do not address the underlying causes of pain (Thiwan, and Drossman, 2006). Additionally, nonsteroidal anti-inflammatory medications (NSAIDs) are frequently used to treat GI tract diseases (Soleimanpour et al., 2016). However, when taken in large dosages and for an extended period of time, these NSAIDS have been shown to produce oral lichen planus, esophageal inflammation and strictures, and small bowel and colonic ulcers (Makins, R., Ballinger, A., 2003). While probiotic treatments are also being introduced to combat GI tract diseases, which involves the ingestion of live microorganisms capable of surviving in the GI tract and exerting a beneficial effect on the host (Whitfield and Shulman, 2009; Pickard et al., 2018). Nonetheless, the efficacy of this biological therapy remains unknown.

With the increasing prevalence of gastrointestinal tract infections, it is vital to study novel sources of natural products capable of targeting disease-causing microorganisms in the GI tract. *Periplaneta americana* or the American cockroach is a synanthropic pest that is primarily found in cosmopolitan to metropolitan regions and thrives in warm, damp climates (Kim et al., 2016). *P. americana* are prevalent in certain areas of Metro Manila (Carillo et al., 2016). Cockroaches in general, are constantly exposed to potentially pathogenic microorganisms and parasites as a result of their ecological roles and habitat (Basseri et al., 2016), with at least 22 species of pathogenic human bacteria, viruses, fungi, protozoans, and five species of helminthic worms isolated from the species (Babara, 2014). Due to their ability to resist these pathogens suggests that *P. americana* could be a potential candidate as source for antimicrobial substances. In recent years, *P. americana* has been for its neurological relevance, particularly in electrophysiology, which plays a critical role in the majority of toxicity research activities (Dabrowski et al., 2012). Meanwhile, an antibiotic assay conducted by Seraj et al., (2003) using a 61kDa protein isolated and purified from *P. americana's* hemolymph revealed an intriguing potential for therapeutic application due to its activity against several bacterial strains at extremely low concentrations in comparison to conventional antibiotics. Similarly, crude lysates of cockroach brain exhibit considerable antibacterial action against MRSA and neuropathogenic *E. coli* K1 (Ali et al., 2017).

To contribute to the search for new antimicrobial sources, the researchers sought to explore a novel source to combat GI tract infectious agents by evaluating the antimicrobial activity of *P. americana* tissue extract as a potential response to the far-reaching complications of GI tract infections worldwide.

Materials and Methods

Collection and Preparation of the Sample

Adult *P. americana* were collected live from four different locations in Metro Manila, the Philippines: Brgy. Concepcion Uno, Marikina City (14. 6507° N, 121.1029° E), Brgy. Punturin, Valenzuela City (14° 44' 13.3260 N, 121 0' 8.5968" E), and Brgy. Addition Hills, Mandaluyong City, Rev. G. Aglipay, Mandaluyong City (14° 35 35. 2903' N, 121° 1.4606 E) (Figure 1).

A total of 160 *P. americana* samples were obtained and stored in a glass container prior to euthanasia; each sample was stripped of its head, pronotum, tegmina, hind wings, legs, and anal cerci. Test strains were procured from the Philippine Network for Microbial Culture Collections (PNMCC), University of the Philippines, Los Baños. The test strains' details are listed in Table 1 along with their designated accession numbers.

Figure 1. Map of sampling area, Metro Manila, Philippines. A. Brgy. Concepcion Uno, Marikina City (14. 6507° N, 121.1029° E), B. Brgy. Punturin, Valenzuela City (14° 44' 13.3260 N, 121 0' 8.5968" E), and C. Brgy. Addition Hills, Mandaluyong City, Rev. G. Aglipay, Mandaluyong City (14° 35 35. 2903' N, 121° 1.4606 E). Photo from Google. (n.d.).

Table 1. List of pathogenic strains used in the experiment

Bacterial Strain	**Accession Number**
Enteroccus faecalis (+)	BIOTECH 10348
Salmonella enteritidis (-)	BIOTECH 1963
Vibrio parahaemolyticus (-)	BIOTECH 10210
Serratia marcescens (-)	BIOTECH 1748
Candida albicans (fungus)	ATCC ® 10231™
** Gram-positive Bacterial Strain (+), Gram-negative Bacterial Strain (-)**	

Solvent Extraction of P. americana Tissue

After the cockroach wings and appendages were removed, the samples were air-dried for three days and then dried further in a laboratory oven. *P. americana* tissue was dehydrated and pulverized in a laboratory blender. Thirty grams of the crushed material were collected and then steeped in ethanol at a 1:3 ratio for three days. After filtering and evaporating the solution, aseptic preservation of the extracts was performed until use for antimicrobial assays (Song et al., 2017).

Kirby-Bauer Disc Diffusion Assay

The Kirby-Bauer Disc Diffusion assay was used to determine the antibacterial activity of the *P. americana* tissue extract. Extracts and conventional antibiotics (Rifampicin, Trimethoprim, Ofloxacin, Penicillin, and Nystatin) were impregnated into previously prepared Mueller-Hinton

Agar plates and incubated for 24 hours at 35-37° Celsius; all assays were performed in triplicate (Sanders, 2012).

Statistical Treatment of Data

The assay generated data, and each parameter was averaged. The Kirby-Bauer Disc Diffusion assay findings were analyzed statistically using one-way ANOVA, with P values less than 0.05 considered significant.

Results and Discussions

Kirby-Bauer Diffusion Assay

A single cockroach species was sampled for tissue extraction and antimicrobial activity assays were determined against indicator microorganisms. The Kirby-Bauer Diffusion assay revealed statistically significant differences in the activity of extracted tissue with the antibiotic controls Ofloxacin, Penicillin, Rifampicin, and Trimethoprim against four bacterial strains and a fungus: *Enterococcus faecalis*, *Salmonella enteritidis*, and *Vibrio parahaemolyticus*, as determined by one-way ANOVA at 0.05. The findings of the experiment are summarized in Table 2.

Table 2. **The average diameter of the zone of inhibitions of extract from *Periplaneta americana***

Pathogens	**Extract**	**Rifampicin**	**Trimethoprim**	**Ofloxacin**	**Penicillin**	**Nystatin**	**p-value**
E. faecalis	-	31.47	31.47	33.6	45	-	0.000
S. enteritidis	-	28.67	31.33	32.67	35.8	-	0.000
V. parahaemolyticus	10.43±0.91	8.8	11.2	25.47	0	-	0.00013[5]
C. albicans	9.27±0.83	-	-	-	-	10.53	0.00091[5]
S. marcescens	-	-	24.83	26.1	-	-	0.000

Note: Diameter of zone of inhibition includes well diameter 8 mm. [5]Refers to statistically significant ≤ 0.05

The high prevalence of gastrointestinal (GI) tract infections caused by *E. faecalis*, *S. enteritidis*, *V. parahaemolyticus*, *Candida albicans*, and *S. marcescens* prompted for the use the test strains in the study. Not only do these microorganisms induce gastrointestinal diseases, they also impose a burden on immunocompromised individuals. As illustrated in the graph, the tissue extract of *P. americana* demonstrated antibacterial activity against two GI pathogens. The findings are consistent with those of other research investigations on the antimicrobial activities of *P. americana* extracts. According to Anwar et al. (2003), a protein isolated from purified hemolymph of *P. americana* inhibited several gram-positive bacteria, but not the gram-negative culture used; in contrast, the *P. americana* tissue extract used in this study exhibited activity against a gram-negative bacterium, *Vibrio parahaemolyticus*. Additionally, the high antibacterial activity of *P. americana* crude brain extract was evaluated in a work pioneered by Ali et al., (2017), with promising results. The cockroach species' potential ability to combat pathogens may be a result of the species' encounters with various microbes and superbugs in their environment, and its ability to fend against sickness through the production of antimicrobial compounds (Ali et al., 2017).

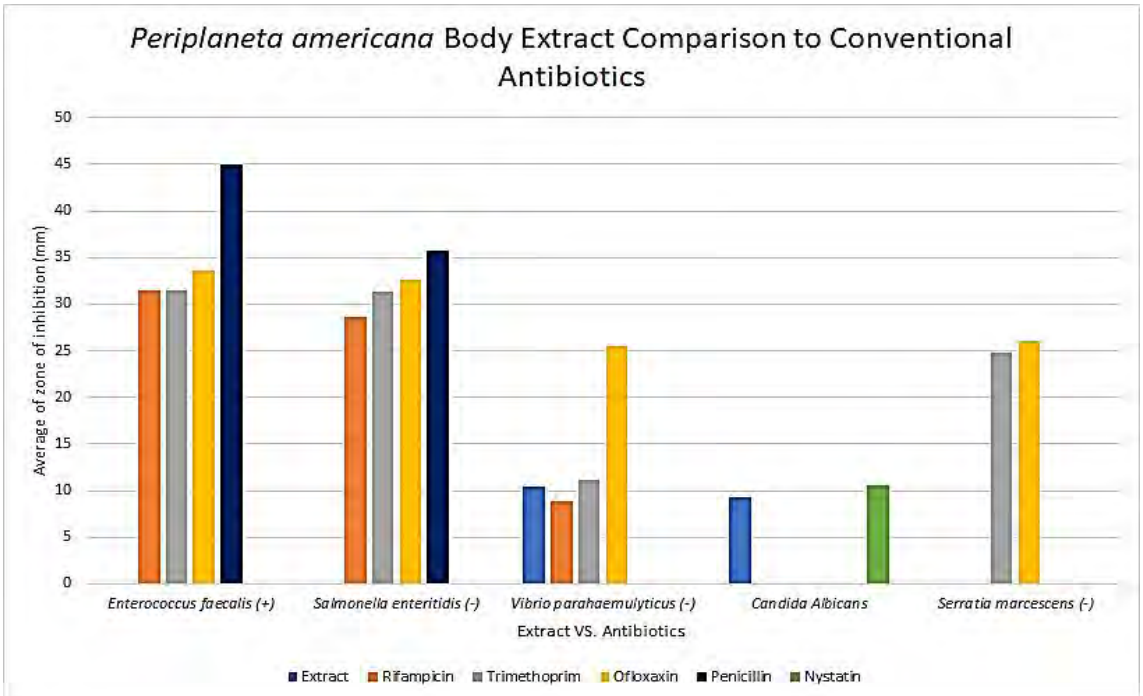

Figure 2. Graph comparing *Periplaneta americana* tissue extract inhibitory activities to five conventional antibiotics: Rifampicin, Trimethoprim, Ofloxacin, Penicillin, and Nystatin; as shown in the graph, the extract inhibited *V. parahaemolyticus* similarly to Rifampicin and Trimethoprim, while Nystatin inhibited *Candida albicans*. There was no inhibition against *E. faecalis, S. enteriditis,* or *S. marcescens*.

The ability of insects to combat or resist bacterial infections is due to their ability to detect bacteria and thus produce a series of antimicrobial peptides that are innately present and released via the hemolymph (Baserri et al., 2016). Antimicrobial peptides are typically composed of small, amphipathic, cationic molecules (Gao and Zhu 2013). According to Ali et al., (2017), few homologous compounds containing isoquinoline groups, chromene derivatives, thiazine groups, imidazoles, pyrrole-containing analogs, sulfonamides, furanones, and flavanones were identified in *Periplaneta americana*. These compounds are known to possess broad-spectrum antimicrobial, anti-inflammatory, anti-tumor, and analgesic properties. Apart from these, *P. americana* was claimed to create inducible antimicrobial peptides, specifically lysozyme, which functions as an opsonin (Kim et al., 2010), an antibody that reacts with invading microorganisms, rendering them susceptible to phagocyte ingestion (Winkelstein, 1973). Subsequently, Kim et al. (2016) discovered that 11 peptides exhibited antibacterial activity against a variety of harmful bacterial strains using a De Novo Transcription Analysis. Further, *P. americana* peptide constituents are designated to contain more than 16 amino acids, including seven human essential amino acids and two human semi-essential amino acids, which increases its possibilities of producing antibiotics (Zhao et al., 2017).

Conclusion

Numerous analyses and experiments on various parts of *P. americana* have demonstrated that it is a promising source of innate antimicrobial components; in this study, the *P. americana* tissue extract was tested for its ability to combat pathogenic enteric bacteria, including *Enterococcus*

faecalis, Salmonella enteriditis, Vibrio parahaemolyticus, and *Candida albicans*. However, the extract was found to be effective only against *V. parahaemolyticus* and *Candida albicans*. The investigation established that *P. americana* is capable of inhibiting gram-negative bacteria, contrary to previous research claims. The antimicrobial activity against test pathogens may be a result of the chemical and biological composition of the *P. americana* tissue. On the other hand, environmental conditions and extraction processes may influence the antimicrobial peptides' potency. Thus, additional study should be undertaken to ascertain the active chemicals included in the extract and to verify the extract's suitability for the creation of new antimicrobials against enteric bacteria and other pathogenic microorganisms.

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