



## In vitro and In silico Antimicrobial Effectiveness of *Persea* extracts against Pathogenic Bacteria and Fungi

Tayal Punitha Ramasamy Naidu<sup>a</sup>, Dr Norazah Binti Basar<sup>b</sup>, Dr Syazwani Itri Binti Amran<sup>c\*</sup>

<sup>a,b</sup>Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor, Malaysia

<sup>c</sup>School of Biomedical Engineering & Health Sciences, Faculty of Engineering,

Universiti Teknologi Malaysia, Johor, Malaysia

\*Corresponding author: syazwaniitri@utm.my

### Abstract

This study was conducted to study the antimicrobial activity of *Persea declinata* and *Persea fruticosa* species. The hydroethanol extract and chloroform, ethyl acetate and butanol fractions of the leaves of *Persea declinata* and *Persea fruticosa* will be studied on selected bacterial and fungi strains such as *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. Both in vitro and in silico antimicrobial activities will be studied. The extracts of *Persea declinata* and *Persea fruticosa* are believed to have promising antimicrobial activities against *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*.

**Keywords:** antimicrobial activity; *Persea declinata*; *Persea fruticosa*; In silico.

### Introduction

In recent decades, where the global population has adapted rapid development in every aspect, herbal medicine has become as an alternative option to prescribed drugs, due to the significant medicinal and economic implications. The community favour for the medicine which can produce high recovery with low side effects. *Persea* plant, or commonly known as avocado, also falls into the list of effective medicinal plants. We are aware that avocado fruit is usually added into the weight loss diet [1]. But many studies have been done that the parts of the plant like the leaves, also produce beneficial medicinal components to human kind [2,3]. Based on the health benefits of Avocado, certain countries have even given recognition to the fruit by adding it in their health promoting information to the public. An independent Australian organization, "The Heart Foundation" certified the fruit as a healthy fruit for heart and used the certification in their advertisements. The Californian Avocado Commission conjoined with the American Dietetic Association and American Heart Association, publicized the fruit as health promoter in press releases [4].

*Persea* is a genus of about 150 species of evergreen trees belonging to Lauraceae. The well-known member of the genus, *Persea americana*, widely cultivated in subtropical regions for its large, edible fruit. The leaves are simple, lanceolate in shape, varying in species, broad and arranged spirally or alternatively on the stems. The fruit is an oval shaped berry [5]. The plant is used in traditional medicine for the treatment of various ailments such as menorrhagia, hypertension, stomach ache, bronchitis, diarrhea and diabetes. Besides, the phytoconstituent of the leaf extracts has exhibited pharmacological activities like vasorelaxant, analgesic and anti-inflammatory, anti-convulsant, anti-viral, wound healing, anti-oxidant, anti-ulcer, anti-hepatotoxic and hypoglycemic activity [6,7].

On the other hand, the methanolic crude extract of *Persea declinata* has been proven to be active on various cancer cells, demonstrating anti-tumor activity against human breast cancer cells via cycle arrest and apoptosis induction process [8]. Another study demonstrated that the leave and stem bark of *Persea declinata* extraction contain reducing sugars, cardiac glycosides, coumarins, steroids, flavonoids, tannins and phenolic compounds. However, the pharmacological reaction of each component was not studied [9].

Moreover, World Health Organization (WHO) recently has declared that antimicrobial resistance (AMR) is one of the top 10 global public health threats being faced by humanity. Overuse and not being compliant when using antibiotic by infectious disease patients, led to the growth of drug-resistant pathogens. The cost of AMR to national economies affects the productivity of patients and their care takers, by prolonging their hospitalization and increasing the expenses [10]. A good quality antimicrobial agent with plant based, would treat the infections with low adverse events. *Persea* with its prominent antimicrobial activity has gained the believe of many patients in various countries for multiple disorders.

Most of the studies on the medicinal activities were done on *Persea americana* species [3,11,12]. There are also few numbers of research papers available on *Persea declinata* species [8,9,13]. However, there is relatively little research on the medicinal components of *Persea fruticosa*. The *Persea* genus is believed to be potential in antimicrobial activities against pathogenic bacteria and fungi. There are more room to be explored in the *Persea* genus, specifically on different parts of the plant like leaves and fruits.

Besides that, different liquid extraction method can help for different phytoconstituent identification. From example, the extraction from water and ethanol possible to produce different phytochemical components. Even though, some studies focused their research on the identification of *Persea* phytochemical components, but their medicinal values were not tested yet. The further research needs to be continued so that it will give wider option for the treatment of various infectious diseases.

For an effective drug discovery, usually high expenses of laboratory work will eventually give a drug with higher cost price. In the intention of overcoming the cost, this study will also include the *in silico* method, one way in reducing the expensive lab work. The *in silico* antimicrobial activity involves digital genetic sequencing. This would be a stronger evidence for the antimicrobial characteristic of *Persea* plant. Eventually, it would be more affordable for the global community to reach the *Persea* based drugs.

Thus, the objectives of the research are:

- a) To assess the potential antimicrobial effectiveness of hydroethanol extract and chloroform, ethyl acetate and butanol fractions of the leaves of different species of *Persea* extracts against different types of pathogenic bacteria and fungi.
- b) *In vitro* and *in silico* antimicrobial activity will be carried out to evaluate the pharmacokinetic and toxicological properties of the targeted phytoconstituent.
- c) To discover specific pathogenic bacteria and fungi which are not resistant to *Persea* extracts.

## Materials and methods

Since most of the studies on the antibacterial activities were done on *Persea americana* species, this study will be focused on antibacterial activity of *Persea declinata* and *Persea fruticosa* species. Below is the methodology will be used for this study.

### 1. Collection and identification of plant materials

The selection criteria of the *Persea* species to be studied will be based on their ethnopharmacology, chemotaxonomy and those may contain the appropriate phytoconstituent which correlates with the objective of this study. *Persea declinata* and *Persea fruticosa* will be focused more in this study since lack of research on their medicinal values. Table 1 is showing the traditional uses of common *Persea* species extracts.

### 2. Extraction

Fresh leaves will be dried in an oven with air circulation at 45°C for 7 days. The dried plant material is extracted with aqueous ethanol (ethanol: water 70:30, v/v) by maceration as described in Deuschle et al. The hydroethanol extract obtained will be further fractionated in separation funnels by sequential extraction using increasingly polar solvents such as chloroform, ethyl acetate and *n*-butanol. The organic fractions will be concentrated and dried in rotary evaporator [14]. This essential oil extracts will be used in the methodology step number 6, the *in vitro* antibacterial and antifungal study.

### 3. TLC analysis

Phytochemical screening of the extract and fractions will be carried out in order to detect secondary metabolites using thin layer chromatography (TLC). After the color elution, results will be observed under UV/vis light and followed by spray using specific reagents (For flavonoids - sulfuric acid anisaldehyde; terpenoids and saponins - vanillin sulfuric acid; alkaloids - Dragendorff reagent) [14]

### 4. HPLC analysis

Reversed - phase chromatograph analyses will be carried out in gradient conditions. Quantification is carried out by integration of the peak using external standard method. The chromatographic peaks will be confirmed by comparing their retention time and DAD-UV spectra with those of the reference standards. All chromatographic operations will be carried out at room temperature ( $25^{\circ}\text{C} \pm 1^{\circ}$ ) and triplicate [14].

### 5. Bacterial strains

Bacterial and fungi strains will be purchased from the nearest hospital (Hospital Sultanah Aminah, Johor Bahru). The possible strains to be involved in this study are *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*. The bacterial stains will be grown in Nutrient Broth medium at  $37^{\circ}\text{C}$  for 8 hours.

### 6. In vitro antibacterial and antifungal study

Inhibition of the bacterial and fungi growth by the plant extracts will be evaluated by disc diffusion assay. The sterile Whatman filter papers are prepared and soaked separately in each of the essential oil extracts for 5 min. The filter papers placed on the plates. After 24 hours of incubation at  $37^{\circ}\text{C}$ , the zone of inhibition around each disc is measured. The MIC values are also determined by micro-dilution method. The plant extracts are serially diluted and added to a 96-well plate. 100 $\mu\text{l}$  of an appropriate medium (Mueller - Hinton Broth) and 20 $\mu\text{l}$  of the inoculums (containing about  $6 \times 10^4$  colonies) are dispensed into each well of a 96 well plate. After 24 hours of incubation period at  $30^{\circ}\text{C}$ , plates are read at optical density (OD) reading at 620 nm using a spectrophotometer for bacteria. MIC value will be defined as the lowest concentration which inhibits the growth or fewer than 3 discrete colonies will be detected. On the other hand, MBC value will be defined as the lowest concentration of the plant extracts to kill the microorganisms. Plates will be read in triplicate and the average MIC value will be recorded [15]

### 7. Molecular docking analysis

Ten active compounds from the extract will be selected. All these compounds are subjected to molecular docking studies for inhibition of antibiotic target proteins. In this study 5 target proteins consist of DNAgyrase subunit B (DGSB with PDB entry 3TTZ), Pincilin binding protein (PBP1a with PDB entry 3UDI), D-alaninD- alanin ligase (Ddl with PDB entry 2ZDQ), dihydrofolate reductase (DHFR with PDB entry 3SRW) and dihydropteroate synthetase (DHPS with PDB entry 2VEG) are chosen for docking study. Besides, molecular docking is performed for 5 standard antibiotics (Cirprofloxacin, Benzylpenicillin, Sulfadiazine, Trimethoprim and Mupirocin) with mentioned target proteins as positive control. The 3D structure of mentioned compounds and standard antibiotics are obtained from Pub Chem (<http://pubchem.ncbi.nlm.nih.gov>) database as SDF format. The 3D structure of mentioned target proteins will be also obtained from the protein data bank as PDB format. Molecular Docking will be performed using Autodock4 with the Lamarckian genetic algorithm. Docked conformations will be clustered by a tolerance of 2A root mean square deviations (RMSD) [15].

### Expected Results

Based on Tosun et al., a plant extract which has MIC reading of below 200 µg/ml, is considered to have an active antimicrobial activity (16). *Persea declinata* and *Persea fruticosa* extracts are expected to have an active antimicrobial activity on *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* at different range of MIC reading values. The results of molecular docking are expected to have appropriate interactions between the studied compounds and the antibiotic targets.

**Table 1:** Different species of *Persea* and their traditional uses.

Scientific Name	Common English Name	Plant Part Used	Traditional Uses
<i>Persea americana</i>	Avocado	Leaves	Dysentery, cough, high blood pressure, liver problems and gout
<i>Persea americana</i>		Bark	Diarrhea
<i>Persea americana</i>		Fruit	To lower blood cholesterol level, promote hair growth, soothe the skin
<i>Persea declinata</i>	Medang Inai		Anticancer activity (Narrima et al., 2014)

### Conclusion

The extracts of *Persea declinata* and *Persea fruticosa* are believed to have promising antimicrobial activity against *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus aureus*, *Vibrio spp.*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*.

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