Evaluation of Antioxidant and Antimicrobial Activity of *Artocarpus altilis* Against Human Pathogens

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**Abstract**

The antioxidant and antimicrobial activities have been performed using methanol fruit extract of *Artocarpus altilis*. Results of the phytochemical screening revealed that methanolic extract of *A. altilis* fruits contain flavonoids, phenols, steroids and glycosides. The investigation on these constituents was done using the phytochemical screening with modification. The soxhlet apparatus was employed to extract the dried plant using methanol as a solvent. It was observed that the extract has characteristics of antioxidant using DPPH assay. The antibacterial and antifungal potential of methanolic fruit extract were studied against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella* spp., *Aspergillus niger* and *Candida albicans*. This test was performed by using disc-diffusion method with different concentrations of fruit extract. It was found that one bacterial species (i.e. *Staphylococcus aureus*) showed inhibition zone with maximum (zone of inhibition≈15mm) using 150 mg/ml of fruit extract. Methanolic extracts of *A. altilis* had no effect on growth of the remaining microorganisms. The phytoconstituents and characteristics of microorganisms might be responsible for the antimicrobial activity of the extract. Further purifications of secondary metabolites and structural studies may lead to the isolation of the active compounds.

1 Introduction

Medicinal plants are the essentials of traditional remedies because they have therapeutic constituents\(^1\)-\(^3\). Plants contain many drugs with different pharmacologic properties\(^4\). A wide range of medicinal plants are used to extract raw drugs in a huge quantity and traded in the market as unrefined material for many herbal manufacturers\(^5\). The search for new antioxidant and antimicrobial agents has increased remarkably due to multiple drug resistance. Oxidative damage in human cells or tissues is mainly due to the uncontrolled production of oxygen from free radicals. Free radicals are harmful substances which produced in the body during the normal metabolic processes. Environmental chemicals such as pesticides and other chemicals like alcohol, cigarette smoking, burning of organic matter, UV radiations and automobile pollution also contribute to free radical generation\(^5\). Bacterial, fungal and viral infections also produce reactive oxygen species. Secondary metabolites presence in plants with antibacterial and antioxidant potentials has been actively investigated as alternatives for the therapy of bacterial infections and many kind of degenerative diseases\(^6\). Among many phytochemical, flavonoids seem to be the most important candidates for this purpose.

Therefore, naturally occurring antioxidants are now the focus of many reasearch studies. Antioxidants are molecules which interact with free radicals and stop the chain reaction before vital molecules (DNA lipid and proteins) are affected\(^7\). The use of synthetic antioxidants has been limited because of their toxicity effects\(^8\). According to the worldwide statistic, infectious disease still holds the leading point in causing mortality and disability\(^5\). The random use of antimicrobial drugs has dragged to multidrug resistance, which has become the major impediment to treat infectious disease. This has drove to a very high demand for the alternative drugs in the treatment of infectious disease from the medicinal plants. Moreover,
the natural antimicrobial have less side effects compared with the synthetic ones10.

Artocarpus altilis from Morocae family has been used as an important traditional medicine in Malaysia and other countries (Fig. 1). Artocarpus altilis is very famous for its nutritional, therapeutics, and medicinal properties. The medical use of Artocarpus altilis is abundant and unlimited. The leaves had been reported to reduce high blood pressure and diabetes and it is believed to treat liver disease and fevers in Taiwan. Moreover, the crushed leaves is known to treat the oral fungus disease and ear infections besides flower extract for ear edema, and the root as a purgative or used (when macerated) as a poultice for skin ailments whereas the bark known to treat headaches. A complex organic acid in the leaf extracts (gamma-amino butyric acid) is the active ingredients which have the relaxing, anti-anxiety, and anti-convulsive effects11,12. The powdered roasted leaves are used for enlarged spleen. In the Pacific Islands the Artocarpus altilis was used as an important staple food. Root and stem bark extracts possessed antimicrobial property as it showed antimicrobial activity against gram-positive bacteria with the capability in treating tumors13. High content of amino acid, fatty acids and carbohydrates were recorded by the chromatographic study of bread fruit leaf and fruit extracts14.

Figure 1 Plant of Artocarpus Altilis

There are many traditional declarations of this plant that is yet to be proven. Thus, the aim of this study was to identify the antibacterial, antifungal as well as antioxidant activity of the extracts of Artocarpus altilis. This study would emphasize on the relationship between phytochemical compounds with the antimicrobial and antioxidants properties of Artocarpus altilis. Despite many synthetic drugs that are available in the market, serves as antioxidants and antimicrobial agents, but natural plants still capable to substitute them with fewer side effects.

2 Materials and Methods

2.1 Study Area

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The research was carried out at the research laboratory one of Management & Science University between the months of November 2013 and January 2014.

2.2 Collection of plant materials

Fruit of Artocarpus altilis was collected from the local garden in Perak, Malaysia. The plants were sent to Forest Research Institute of Malaysia (FRIM), Kepong, Selangor, Malaysia for identification and authentication of plant. Vouchers specimens of fruit of A. altilis were obtained from Herbarium of FRIM (PID 260613-14).

2.3 Preparation of plant extracts

The fruits of Artocarpus altilis were collected and kept for air dried; and reduced into coarsely powder. The coarsely powdered fruit was macerated with 100% methanol. The extracts were filtered using Watmann Sterile filter paper and concentrated using rotary vacuum evaporator under reduced pressure. The concentrated extracts were kept in air tight container until use.

2.4 Preliminary phytoconstituents screening

The extracts of Artocarpus altilis were subjected to phytochemical studies to investigate the presence of phytoconstituents such as flavonoids, phenolic group, tannins, saponins, glycosides, alkaloids and steroids.

2.5 Determination of DPPH radical scavenging activity assay

The extracts of 1.0 ml, contained 1-200 µg of dry matter per 1 ml of methanol mixed with 1 ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) solutions. These were further diluted with 95% methanol to a final volume of 4 ml. The resulting extract solutions, and a blank were kept at room temperature for 1 hour, and absorbance of solution was measured at 515 nm using a spectrophotometer. The synthetic antioxidants, butylated hydroxytoluene (BHT) were used as positive control. The percentages of free radical DPPH scavenging were calculated according to the following relationship:

\[
\% \text{ DPPH scavenging} = 100 \times \frac{\text{blank} - \text{sample}}{\text{blank}}
\]

Where, blank was the absorbance of the control reaction mixture excluded the test compounds and sample was the absorbance of test compounds. IC_{50} values which correspond to the concentration of sample extracts that caused a 50% neutralization of DPPH, were calculated from the plotted of percent DPPH scavenging versus concentration15-17.

2.6 Collection of microorganisms and microbial cultures

The cultures of different bacterial strains (Staphylococcus aureus, Klebsiella pneumonia, and Salmonella spp.) was obtained from microbiology laboratory of Management & Science University, and maintained at 50°C. Broth cultures of the microbial strains showing UK J Pharm & Biosci, 2014: 2(4); 11
an absorbance value ranging between 0.129-0.134 at a wavelength of 517 nm were used for testing antimicrobial activity.

2.7 Determination of antibacterial activity

The extracts were subjected to antibacterial activity using modified disc diffusion method. Mueller Hinton Agar was used to culture the bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella spp.*). The bacterial suspension was spread uniformly on the solid agar medium using cotton swab. Sterile Watmann filter paper disc with diameter of 6 mm was impregnated with 10ul of extract of different concentrations (50 mg/ml), (100 mg/ml), (150 mg/ml) of fruit extract and placed on the upper layer of inoculated agar medium. The standard 6mm disc of Amoxicillin (10 µg/disc) were used as positive control whereas 10 µl of DMSO as negative control. The seeded agar plate were dried for 15 minutes and incubated at 37 °C for 24 hours. The antibacterial activity was assessed by measuring the diameter of inhibition zone.

2.8 Determination of antifungal activity

The extracts were tested for antibacterial activity using modified disc diffusion method. Sabourad agar was used to culture the fungi (*Candida albicans*, *Aspergillus niger* and *Penicillium notatum*). The fungal suspension was spread uniformly on the solid agar medium using cotton swab. Sterile Watmann filter paper disc with diameter of 6mm was impregnated with 10ul of extract of different concentrations (50 mg/ml), (100 mg/ml), (150 mg/ml) of fruit extract and placed on the upper layer of the inoculated agar medium. The standard 6 mm disc of Fluconazole (30 µg/disc) were used as positive control whereas 10 µl of DMSO as negative control. The seeded agar plate were dried for 15 minutes and incubated at 37 °C for 72 hours. The antifungal activity was assessed by measuring the diameter of inhibition zone.

2.9 Statistical analysis

Data are expressed as mean ± SEM of the triplicates. The scavenging activity of fruit extract and standard (BHT) as well as the antimicrobial activity of the fruit extracts, and standard antimicrobial agents (drugs) was compared using T-Test by SPSS version 20.

3 Results

The result of the phytochemical screening of *A. altilis* presented in Table 1, confirmed the presence of various metabolites in the fruit. The methanolic extract of *A. altilis* exhibited the presence of phenolic compound, flavonoids, glycosides and steroids. The presence of flavonoids was observed when the configuration of yellow after the addition of lead acetate solution. Upon the addition of gelatin reagent, the appearance of a white precipitate is an indicator of the presence of phenolic groups. A reddish brown colour formed at the correlation of the two layers indicated the positive result of the presence of glycosides.

Table 1 Phytochemical screening test of *Artocarpus altilis* (fruit)

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic group</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
</tbody>
</table>

The results of antioxidant activity were expressed as IC\textsubscript{50} value, which is defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals of the plant fruits extract and the standard (positive control). In this study, the methanol extract of *A. altilis* showed a remarkable antioxidant activity. The comparative result of IC\textsubscript{50} between standard and extracts are listed in Table 2 (Fig 2).

Table 2 DPPH radical scavenging activity (IC\textsubscript{50}) of the control, plant’s fruit extracts and standard

<table>
<thead>
<tr>
<th>Samples</th>
<th>Extracts</th>
<th>IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100% Methanol</td>
<td>0</td>
</tr>
<tr>
<td><em>A. Altilis</em></td>
<td>100% Methanol</td>
<td>5.736</td>
</tr>
<tr>
<td>BHT</td>
<td>100% Methanol</td>
<td>10.729</td>
</tr>
</tbody>
</table>

Methanolic fruits extract of *Artocarpus altilis* was effective only against *Staphylococcus aureus* in comparison to other pathogenic bacterial and fungal used in this study as shown by the presence of zone of inhibition.

4 Discussion

Preliminary phytochemical screening showed the presence of flavonoids, phenols, steroids and glycoside in methanolic extract of *A. altilis* fruits. It was supported by earlier reports for the presences of phenols and flavonoids in the Morocae family. Flavonoids and phenol exhibit a wide range of biological activities, one of which is
they have the properties of antioxidant and antimicrobial activity. It was noticed that the extract has antioxidant effect using DPPH assay. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. IC₅₀ value was used to express the activity of antioxidant activity. IC₅₀ is defined as the concentration of antioxidant for 50% scavenging of DPPH radicals of the plant and the standard (BHT). Furthermore, the lower the IC₅₀ value, the higher the antioxidant activity of the extract, and this result was obtained by the 100% methanol extract with IC₅₀ value of 5.736 µg/ml.

Figure 2 Scavenging activities of the methanolic extract of the *Artocarpus altillis* fruit and standard

Methanolic fruit extract of *A. altillis* was effective against *Staphylococcus aureus* in comparison to other pathogens used in this study. Maximum inhibition activity of *Staphylococcus aureus* (Zone of Inhibition: 15mm) was noticed with the 10µl methanolic fruit extract of *A. altillis* (≈150 mg dry fruit matter) (Fig 3). These results show the efficacy of methanolic fruit extract of *A. altillis* against gram positive bacteria *Staphylococcus aureus* probably due to the presence of phytoconstituents such as flavonoids, sterols and phenols. This result may also associated with the characteristics of gram positive *Staphylococcus aureus* which is less resistance to physical disruption and more susceptible to antibiotics. The remaining pathogens used in this test which is gram negative bacteria *Salmonella* and *Klebsiella pneumonia*, as well as fungus, *Candida albicans*, *Penicillium notatum* and *Aspergillus niger* showed negligible zone of inhibition in all triplicate tests.

The negative result for antimicrobial and antifungal against all the remaining pathogens may be attributed to the absence of certain phytochemical constituents like Tannins. Tannins show the antimicrobial activity by precipitating the microbial proteins. Tannins also react with proteins and act as stable and potent antioxidants which fights against various toxins released from the microbes¹⁹,²⁰.

Figure 3 Methanolic fruit extracts showed inhibition growth of *S. aureus*

5 Conclusion

The results of present investigation revealed that the methanolic fruit extract of *Artocarpus altillis* has clearly indicates the characteristics of antioxidant properties. The result showed good source of phytoconstituents. Flavonoids and phenols play a vital role in scavenging the free radicals and these are the phytoconstituents to be focused on for investigation of many biological activities. However, the findings was not enough to support that this extract has a potential antibacterial and antifungal activities against selected pathogens as it only inhibits the growth of *Staphylococcus aureus*. The absences of tannins and saponins are one of the contributing factors for the antibacterial activities of extract. Such phytochemical compounds are needed to make the extracts more potent to fight against those microorganisms. Further purifications can aid in the isolation of active compounds from this plant.

6 Acknowledgements

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7 References


