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# Phytochemical constituents, antioxidant, antibacterial and cytotoxicity properties of *Pandanus tectorius*

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ABSTRACT
Our aim of this study was to do a detailed evaluation of *Pandanus tectorius*the antioxidant, antibacterial,phytochemical constituents and Disk diffusioncytotoxicity properties of *Pandanustectorius*, which in DPPH commonEnglish known as thatch screwpine or pandanus.MCF-7 cell lineThe fruit got a common name as Hala Fruit. DPPH (1,1- diphenyl-2-picrylhydrazyl) method,the most widely reported free radical scavenging activity was used to measure and determine the antioxidant activity. Disc diffusion method was used to measure the antibacterial activity and the cytotoxicity was measured by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS). The result showed that seed extracts had a higher level of phenolics, while leaves extract possessed higher flavonoid content. Seed extracts showed the highest antioxidant activity, strong antibacterial activity, and no cytotoxic activity. Therefore looking at the test result, a clear inference can be drawn that *Pandanustectorius* seeds have excellent characteristics to act as an antibacterial and antioxidant agent.
**Keywords:** antibacterial activity, antioxidant, cytotoxicity properties
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## 1. INTRODUCTION

The role of any medicinal plant in our day to day life is of supreme importance and is effectively used to cure several diseases in a natural way. Modern days, there is a shift of paradigm where it is clearly understood that medicines derived from plants are relatively safer and cost-effective (Dumaoal *et al*, 2010).

*Pandanus tectorius* is one such important plant with phenomenal medical properties. According to Gallaher (2014), the evolution of *Pandanus tectorius* took place approximately two to four million years ago along the coast of Queensland in Australia from a group of species inhabited along the coastal areas. More than 700 species are spread out across the world over tropical and subtropical regimen (Tan *et al*, 2008). Ayurveda practitioners or commonly known as flock medical practitioners have given supreme importance to this medicinal plant as every part of this plant has proven medicinal properties (Rosario and Esguerra, 2003). The leaves contain certain essential oil, flavonoid and alkaloids increasing its medicinal properties to many folds (Kumar and Sanjeeva, 2011).

There are several diseases which are treated using this plant leaf, like a headache, smallpox, leprosy, scabies, heart, and brain diseases, earaches, and rheumatic pains, reduce inflammation from arthritis, epilepsy, infertility and urinary disorders (Majundar *et al*, 2006); The fruit extracts are rich with antioxidant compounds like Caffeoylquinic acid, Vitamin C (ascorbic acid), Provitamin-A, Thiamine, Riboflavin, Niacin (Vitamin B3) and β carotene (Englberger *et al*, 2006). It also consists of essential oils like dimethyl acetate, cinnamates and isopentenyl (Vahirua-Lechat *et al*, 2016). The stem barks of this plant is rich with steroids and the root extracts contains benzofuran derivative α terpenoid, β(Beta) carotene, β(Beta) sitosterol, benzyl benzoate, pinoresinol, germacrene-B, vitamin C, viridine, tangerine, and sanidine, (Venkantesh *etal*, 2012) which has rich medicinal values.

Several researchers reported the medicinal importance of *Pandanus tectorius* as hepatotoxic (Londokar and Kemble, 2011), anticancer (Zhang *et al*, 2013), anti-inflammatory (Londokar *et al*, 2010), antiepileptic (Adhar and Bhaskar, 2014), anthelmintic activity (Shankar and Kumar, 2011) and diuretic activity (Rajeswari *et al*, 2012).

There are limited research conducted on the seed of *Pandanus tectorius* as the main focus by the researchers so far has been on the leaves, stem, roots, and fruits. Therefore, to evaluate phytochemical constituents, antioxidant, antibacterial and cytotoxicity of *Pandanus tectorius* leaves, fruits and seeds extracts against MCF-7 cell line, this study was conducted.

## 2. MATERIALS AND METHODS :

### 2.1 *Plant Materials and extractions*

Fresh part of *Pandanustectorius*, such as leaves, fruits, and seeds was collected from Sindangkerta beach, Tasikmalaya, West Java. The fruit was collected in a mature condition that indicates from the orange color of exoderm. The leaves are sampled based on uniformity of size and color. Fruit mesoderm was separated from seeds. Leaves, fruit, and seed cut it small and dried in the shade for seven days. Dried leaves, fruits, and seeds were ground to powder. In a conical flask, the methanol extracts are prepared by mixing 100 gram of leaves, fruit or seed powder with 1 liter of absolute methanol and leaving it for 72 hours at about 25 Degree Celsius temperature. Whatman filter paper was used to filter the mixtures and get the extract which was further dried using a rotary evaporator and finally stored at 4 degrees C.

The herbarium was prepared and a specimen was further identified and authenticated by Dr. Sri EndartiRahayu, MSi, Associate Professor, UniversitasNasional, Jakarta, Indonesia.

### 2.2 *Phytochemical screening*

Different chemical tests were conducted on the methanolic extract of leaves, fruits and seeds of *Pandanustectorius* in order to detect different phytoconstituents using standard procedure (Harborne, 1973; Sofowara, 1993; and Trease and Evans, 1989).

A).Flavonoids Test: During this laboratory test, when concentrated Sulphuric Acid (H2SO4) and 5 ml of ammonia solution were added together to a portion of the fruit, seed and leave crude extract of *Pandanustectorius*, a temporary yellow color formation was observed confirming the presence of flavonoids.

B).Alkaloids Test: During this laboratory test of mixing the crude exact and Wagner's reagent together, a red-brown color residual is left out which confirms the existence of alkaloid.

C).Tannins Test: During this laboratory test on mixing the crude exact with 2 ml of 2% Ferric Chloride, a change in color is observed due to the chemical reaction initially from bluish green and finally the mixture turned black in color which confirmed the existence of tannins.

D).Saponins Test: During this laboratory test on mixing the crude exact with distilled water (approx. 5-mile liter) in a clinical tube and given a vigorous shake, it was observed that it formed a foam which confirmed that saponin exists in it.

E). Quinones Test: During this laboratory test on mixing dilute NaOH with 1 ml of the crude exact, blue-green or red color formation was observed, indicating the presence of quinones.

F). Steroids Test: During this laboratory test on adding acetic anhydride (2ml) to 0.5 ml of the crude extract and adding concentrated H2SO4 to the mixture, a color change phenomenon is observed from violet to blue or green, indicates the presence of steroids.

### 2.3 *Quantitative phytochemical analysis*

#### *2.3.1 Determination of total flavonoid content (Aiyegoro & Okoh, 2010)*

In order to get the findings about the total flavonoid content, the most popular aluminum chloride - colorimetric method was used in which plant extract measuring 1 mile liter, methanol measuring 3 mile liter, aluminum chloride measuring 0.2 mile liter, potassium acetate measuring 0.2 mile liter and distilled water measuring 5.6 mile liter were mixed together and left for thirty minutes under observation at room temperature. The standard curve was used to determine the flavonoid content and was expressed as quercetin equivalent (Quercetin – A standard 1 mg/ml of the extracted compound).

#### *2.3.2 Determination of total phenol content (Aiyegoro & Okoh, 2010)*

In order to get the findings of the total phenol content present in the methanol extract of the leaves, fruits, and seeds; Folin-Ciocalteau reagent method was used with some modification. In this experiment, Folin-Ciocalteau reagent, measuring 2.5 ml of 10%, a 2-mile liter of Na2CO3 solution and a 1-mile liter of the crude plant extract mixed together and kept for development at room temperature for 15 minutes. The standard curve was used to determine the result and gallic-acid was used as a standard.

### 2.3 *Antioxidant*

Brand-Williams *et al* (1995) had described a method which was used with slight modification, to measure the antioxidant activity of the crude extract. During this experiment, plant extract measuring 1-mile liter and DPPH solution measuring 1-mile liter of a 0.1-mile meter in methanol was mixed together to prepare a sample. In order to find out the measurement for the decrease in absorbance at 517 nm after 30 minutes, UV – Vis Spectrophotometer was used. The formula was used to calculate the inhibition percentage is mentioned below:

$$\text{Inhibition \%} = \frac{\text{A blank - A sample}}{\text{A blank}} \times 100\%$$

### 2.4 *Antibacterial activity*

In order to determine the antibacterial activity, widely used disc diffusion method was used (Bauer *et al*, 1966). *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonasaeruginosa*, and *Streptococcus species* were the four organisms used in this study. Isolated subculturing into selected culturing media was done to confirm their identity and ensuring of purity. Testing of the strains was done on the Mueller Hinton Agar (MHA) (Merck, Germany) and stored at 40C. Before being used in the antibacterial assay described below, overnight culturing was carried out at 370C.

In order to prepare the methanolic extract of *Pandanustectorius*, crude extract measuring about 100-mile gram was dissolved in a 1-mile liter of methanol. Further a filter paper disk ( Whatman Filter Paper) of approximately 0.6-mile meter in diameter was taken and approximately 20 µl of the sample mixture was put on it and dried to remove all the solvent of the solution. Further, the bacteria suspension were

spread evenly with the help of cotton and swab into the Mueller Hinton Agar (MHA). Methanol was used as negative control and Ampicillin used as positive control. After incubation for 24 hours at 370C, visible inhibition zone around the disc was observed which indicated clearly the presence of antibacterial activity.

2.5 *Cytotoxicity testing*

2.5.1 Materials

The media used to do the cytotoxicity testing was DMEM which contains almost four times high concentrated amino acid and vitamins. DMEM original composition was 1000 milligram/Liter of glucose, however, optimal results were achieved in certain cell cultivation, when 4500 milligram/Liter of glucose was used. From Promega, the cell proliferation assay kit, cell tiler 96 Aqueous one solution was purchased consisting of {3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)} ( Promega, Madison, WI).

2.5.2 Cell and culture conditions

The MCF – 7 cells obtained from The Integrated Laboratory of Medical Faculty of Indonesian University were grown in DMEM media up to 10% v/v FBS and further incubated at 37 degrees Centigrade in the humidified atmosphere, containing 5% Carbon dioxide.

2.5.3 Cytotoxicity assay

The fruits, leaves and the extracts of the seeds were dissolved in DMSO as a first step of the cytotoxicity testing, further DMSO testing was done at different concentration and it was determined that cytotoxic effect on the MCF-7 cells were negative for concentration up to 2% v/v, the same inference was in alignment to what was written in the literature (Yang *et al*, 2005). For each concentration, three replicates were performed by mixing dilutions of plant extracts ranging from 10-5000µg/ml along with a final volume of 100 µl per well. At a humidified atmosphere containing 55 CO2, plates were incubated at 37 degrees C for 24 hours after that on a confocal fluorescent microscope (Zeis) at 100 X and 400 X magnifications, cells were inspected and examined by general morphology, detachment, and cell lysis to find signs of cytotoxicity. To detect the results, ELISA plate reader was used and measurement of absorbance was done at 490 nm.

The percentage of cell visibility was calculated by using the following :

$$\frac{\text{Abs sample}}{\text{Abs blank}} \times 100$$

$$\% \text{ inhibition} = \frac{(\text{cell control abs} - \text{treatment abs})}{\text{Cell control abs}} \times 100\%$$

Probit analysis calculation was done to find out the values of IC50, ANOVA, and LSD if the findings show a value above 30 µg/mile liter, the cell viability calculation was done using below formula ( Ref Andriani*et al*,2011) criteria of nontoxic activity for the IC50 value of the sample)

$$CV = \frac{\text{average abs of sample}}{\text{average abs of control}} \times 100$$

## 3.RESULTS AND DISCUSSION

3.1 Phytochemical analysis

There were several published reports on the phytochemical composition and antimicrobial properties of the plant parts, viz, stem, root, flower, fruits and leaves, however, reports on phytochemical composition, antibacterial, antioxidant and cytotoxicity of the seed extracts were limited. In this study, we had broadly reported the phytochemical constituents of leaves, fruits and seeds of *Pandanustectorius* along with their antioxidant, antibacterial and cytotoxicity properties.

The Phytochemical studies that were conducted on leaves, fruits, and seeds extract of *Pandanustectorius* indicated clearly the presence of flavonoid, tannin, saponin, quinone, and steroid, as shown below in Table 1, however, these results contrasted with the findings of Kumar and Sanjeva (2011) report. Several other factors can also influence the Phytochemical contents such as genetic, environmental, a degree of maturity at the time of harvest and geographical factors according to Gajenwalaand Gupta (2013).

**Table 1. Phytochemical analysis of. *Pandanustectorius* leaves, fruit and seed extract.**

| Sample | Phytochemical test | | | | | | |
|---|---|---|---|---|---|---|---|
| | Flavonoid | Alkaloid | Tannin | Saponin | quinon | steroid | triterpenoid |
| Leaves | + | - | + | + | - | + | - |
| Fruits | + | - | + | - | - | - | - |
| Seeds | + | - | + | - | + | - | - |

The above table clearly depicting the test results for the presence of flavonoid, alkaloid, tannin, saponin, quinone, steroid and triterpenoid in leaves, fruits, and seeds of *Pandanustectorius* and a clear correlation can be seen between the phytochemical property and the bioactivity property to exhibit medicinal activities.

The hydroxylated phenolic substances, flavonoids, which are the hydroxylated phenolic substances are synthesized by plants in response to microbial protection having powerful anticancer and antioxidant activities (Okwu, 2004).

Tannins can produce rich protein and enhance protein synthesis (Marjorie, 1996), and saponin can help reduction of inflammation (Just *et al*, 1998) and can coagulate red blood cells. Reports confirm that steroids have antibacterial properties (Raquel, 2007).

Reduced quinones are phenols widely used as anticancer agents, antibiotics and antimalarial drugs (O'Brien, 1991). All of these molecules play an integral role in the cytotoxicity associated with the hypersensitivity response of plants against microbial pathogens (Hammond-Kosack and Jones, 1996).

3.2 Total phenolic content

Table 2 represents the Total phenolic content consisting of the methanol extracts, showing the quantity of Flavonoid and Phenolics present in the fruit, leaf and seed extract.

**Table 2. Phenolic content of methanolic extracts of leaves, fruits, and seeds of *Pandanustectorius***

| Phytoconstituents | Plant parts | | |
|---|---|---|---|
| | Leaves | fruits | seeds |
| Phenolics (mg GAE/g) | 77,92 | 70,55 | 118, 17 |
| Flavonoid (mgQE/g) | 12,1 | 3,9 | 8,5 |

Significant variations can be observed from the above result as the content of phenolics having above eight thousand structures are more in the Seed extracts in comparison with the Leaves and Fruits. The phenolic extracts were soluble in the solvent and can easily form hydrogen bonds with the active sites of enzymes and are highly reactive in nature (Rasouli*et al,* 2009).

Phenolic content of the plant part is often correlated with their strong antioxidant activities (Sim*et al*, 2010).

3.3 ***Antioxidant property***

The DPPH radical scavenging activity results are shown in Table 1.

**Table 3. the IC50 values of DPPH scavenging effect of *Pandanustectorius* extracts**

| Plantextracts | IC50 (DPPH) |
|---|---|
| Leaves | 112 µg/ml |
| Fruit | 158 µg/ml |
| Seeds | 41 µg/ml |

The statistics as shared in Table 3 are evident to conclude that the scavenging effects of leaves, fruits and seed extract on DPPH radicals are excellent, however, leaves and fruits revealed a low value of antioxidant value, However, the best antioxidant properties (IC50 = 41 µg/ml) are revealed by the seeds in comparison with the leaves and fruits, which is because of a large amount of Phenolics compound present in the Seeds (Table 2).

Flavonoids are one among the Phenolic compounds categories with a high amount of antioxidant and have several positive effects on human health,however, when radical scavenging of leaf extract was done, the findings show that they have high flavonoid content with poor antioxidant activity. Variations were also noted in the molecular antioxidant response of phenolic compounds due to their chemical structure or because of the presence of another chemical component present in the extract, such as sugars or ascorbic acid (Singleton and Rossi, 1965).

3.4 ***Antibacterial activity***

A popular method, named as Paper-disc-diffusion was used to determine the antibacterial activity of the *Pandanustectorius*, where evaluation of extracts was done against two Gram positives (*S.aureus* and *Streptococcus* sp.) and two Gram-negatives (*E.coli* and *P. aeruginosa*). On receiving the results (Table 3), it was clearly evident that only the seed extract of *Pseudomonas aeruginosa* delivered the best result in comparison with the others.

**Table 4 Antibacterial activity of crude extracts of *Pandanustectorius*:**

| Inhibition diameter (mm) | | | | |
|---|---|---|---|---|
| Sample/Standard | *E.coliP. aeruginosa* | | *S. aureus* | *Streptococcus* sp. |
| Leaves | 7.3 | 7.4 | 7.1 | 7.3 |
| Fruits | 8.5 | 7.9 | 7.3 | 8.2 |
| Seeds | 7.1 | 13.1 | 7.0 | 8.4 |
| Ampicilin | 19 | 19 | 20 | 19 |

Variability in antimicrobial activity between different plant part extract which could be because of the presence of different chemical compound present in these plant (Noumeden *et al*, 2013). In our study, this activity seemed to be influenced by the phenolic composition and this is in accordance with previous studies that demonstrate a significant correlation between phenolic composition and antimicrobial activity (Bahri-Sahloul *et al*, 2014).

### 3.5. *In vitro* Assay for Cytotoxic Activity

There was no promising cytotoxic effects seen from the *In vitro* cytotoxic assay from the methanolic extracts of the leaves, fruits and seeds of *Pandanus tectorius* and these data are in co-relation with the research reports of Andriani *et al* (2015) where it was reported that the core parts of the fruits do not have any cytotoxic against MCF-7 cell lines.

Our result demonstrate lower cytotoxicity effect of *Pandanustectorius* leaves, fruits and seed extract against MCF-7 cell line by MTS Assay (i.e 2900 µg/ ml, 2500 µg/ ml and 1200 µg/ ml) for leaves, fruits and seed extract respectively (Figure 1) as compared to previous research showed that ethanolic leaf and fruit extract (IC values 105.09 µg/ ml and 773.69 µg/ ml respectively) to generate lower IC50 against T47D cell line by MTT assay (Mukhlis *et al*, 2013).

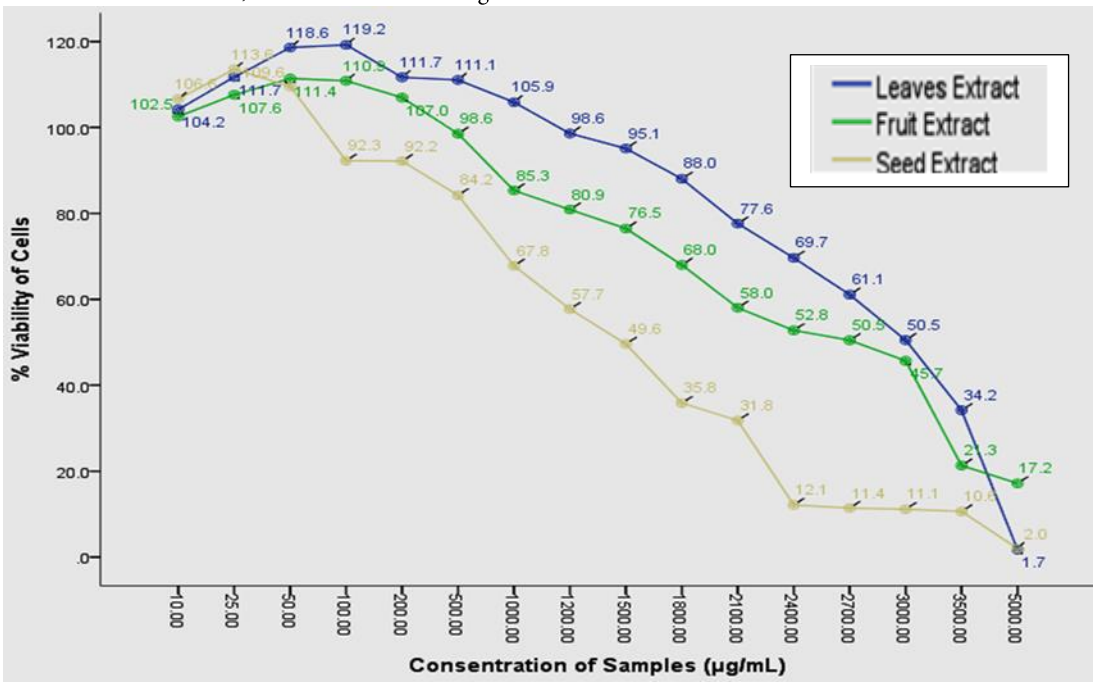


**Figure 1. Cytotoxicity property of*Pandanustectorius*fruits, leaves and seed extracts against MCF-7 cell lines**

Dietary flavonoids act as an anti-cancer agent and its role in the treatment and prevention of cancer is of supreme importance and discussed at length. Several reports were already submitted depicting the role of phenolic and flavonoids compound in cancer treatment, as they act as anti-cancer agents.

Our current research on *Pandanustectorius* seeds depicts that the seeds did not show any cytotoxicity property against MCF-7 cell, but are rich with flavonoids and phenolics along with high antioxidant property which could be an antagonistic effect between chemical constituents in *Pandanustectorius* seeds blocking anticancer potency or cytotoxic effect of extracts against MCF-7 cells,

## 3. CONCLUSION

This is the first comprehensive study of the antioxidant, antibacterial and cytotoxicity properties of *Pandanustectorius* seeds extracts and an attempt was made to explore the diverse

phytochemical efficiency. The various findings and results can be summarized and concluded that *Pandanustectorius* seed extract contains high phenolic content and chemical constituents like flavonoid, tannin, and quinone, resulting in their strong antioxidant capacity and antibacterial activity. The seed of this plant could be a potential source of natural antioxidant having greater importance as therapeutic agents in the treatment of anti-aging and stress.

**Conflict of interest statement**

We hereby declare that there is no conflict of interest regarding the publication of this paper.

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Appendix A.

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