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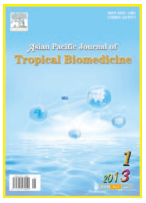

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Antioxidant and cytotoxic activity of *Acanthus ilicifolius* flower

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PEER REVIEW

Peer reviewer

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Comments

The article is explained the best method to extract the active compounds from *A. ilicifolius* that have high antioxidant activity. Moreover the extracts have anti-cancer activity with low dose that warrant cancer therapeutic. This finding is really important for community, scientist and pharmaceutical company as frontier information to explore *A. ilicifolius* as source of anti-cancer material.

(Details on Page 20)

ABSTRACT

Objective: To investigate the antioxidant and cytotoxic activity of the flower of *Acanthus ilicifolius* (*A. ilicifolius*). **Methods:** Antioxidant activity was determined as antiradical efficiency with diphenyl picrylhydrazil (DPPH) method and cytotoxic assay was undertaken using brine shrimp lethal toxicity test. **Results:** *A. ilicifolius* flower contained terpenoid, phenolic compounds, and alkaloid. The methanol extract of *A. ilicifolius* flower showed the highest antiradical efficiency (AE=1.41×10^{-3}) against DPPH radicals and the highest cytotoxicity (LC_{50}=22 μg/mL) against brine shrimp nauplii. **Conclusions:** It is suggested that active compounds of *A. ilicifolius* flower solved in methanol play a role to inhibit free radical activity and kill *Artemia salina* nauplii. The substances can be considered as potential antioxidant and cytotoxic agents as well as imminent candidate for cancer therapy.

KEYWORDS

Acanthus ilicifolius, Antioxidant, Cytotoxic, Flower

1. Introduction

Cancer is a notorious disease that now becomes the major cause of human mortality in the world. Almost half of the incidence and mortality happen in Asia, with lung and bronchus, breast, and colorectal cancers in women to be the most common fatal cancers[1]. The aetiology of cancer is primarily from unhealthy lifestyle and pollution. Carcinogenesis or production of cancer involves mainly three steps, namely initiation, promotion, and progression. The implication of free radicals in different steps of carcinogenesis is well documented. Some of the reactive oxygen and nitrogen species and their pathways could facilitate cancer development by DNA or other biomolecules damaging[2].

Chemotherapeutic drugs are still considered as the most important treatments for cancer, however, this kind of treatments trigger enormous side effects. Regarding this dilemma, ongoing research on natural medicine sources in form of functional foods or nutraceuticals has been attracting many scientists. Phytochemicals containing antioxidant properties showed capacity to

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inhibit carcinogenesis[3]. Several studies discovered these antioxidant compounds are related to other bioactivities. For instances, *in-vitro* cytotoxicity in tumor cells[4], *in-vivo* cytotoxicity in experimental animals and anti-carcinogenesis[5,6].

There are numerous mechanisms of antioxidant substances as anticancer such as: by scavenging free radical[7], by inducing antioxidant enzymes[8], by modulating protein kinase and lipid kinase signaling pathway[9], by inhibiting cyclooxygenase-2 (COX-2) enzymatic activity[10], by affecting phytoestrogenic[11], by influencing nuclear transcription factor NF-κB[12], by inducting phase I or phase II metabolizing enzymes[13], inducting cell cycle arrest[14], and by inhibiting matrix metalloproteinases (MMP)-2 and MMP-9[15].

Based on World Health Organization data, more than 80% of world inhabitants depend on using plant for their medicine and mangroves have been widely used for that purpose[16,17]. *Acanthus ilicifolius (A. ilicifolius)* is a mangrove species that has been utilized traditionally for human remedies. Fruit, leaves, bark, and root of *A. ilicifolius* have been used for asthma, diabetes, hepatitis, inflammation, and rheumatoid treatments[17,20]. This mangrove species has been known to contain bioactive compounds *e.g.* triterpenoids, alkaloids, phenolic compounds, lignan, flavonoid, steroids, and terpenoids[21]. The leaf, root, stem, and bark of *A. ilicifolius* have been reported to be able to prevent tumor growth and cancer progression[22,23]. In order to intensively explore the potency of *A. ilicifolius* for cancer medication, the study of antioxidant and cytotoxic activity of *A. ilicifolius* flower was undertaken.

2. Materials and methods

2.1. Collection of plant samples

A. ilicifolius KŐENIG flowers were collected in November 2010 from Tempurejo village in Surabaya District of East Java, Indonesia and authenticated by the botanist from Department of Botany, Brawijaya University.

2.2. Phytochemical screening

Fresh *A. ilicifolius* flower were air dried and then homogenized into fine powder and stored in airtight bottles at 4 °C . Qualitative phytochemical analysis of the flower powder was done based on Farnsworth *et al.* method[24].

2.3. Preparation of flower extract of A. ilicifolius

The flower extract of *A. ilicifolius* was prepared by maceration. Around 100 g of fresh flower samples were macerated in 300 mL of acetone *p.a.*, methanol *p.a.*, acetone 70%, methanol 80%, and aquades, respectively. The maceration procedure was repeated three times for 24 h. The extract was then filtered using Whatman filter paper. The filtrate was vacuum-evaporated and freeze dried before it was used for antioxidant and cytotoxic assay.

2.4. Antioxidant analysis

The antioxidant activity of flower extract was examined using diphenyl picril hydrazil (DPPH) radical[25]. The 0.5 mmol DPPH was obtained by dissolving it with methanol. One mL of serial concentration of extract diluted in methanol was mixed in 3 mL of DPPH solution and then read their absorbance on spectrophotometer at 515 nm. Effective concentration (EC_{50}) and time of effective concentration (T_{EC50}) were analysed using statistic software of GraphPad Prism 5.0 and Excel 4.0. The antiradical efficiency was determined using the following equation:

$$AE = \frac{1}{EC_{50} \times T_{EC50}}$$

where: EC_{50}: concentration to reduction 50 percent of free radical, T_{EC50}: time needed to reach the EC_{50}

2.5. Cytotoxic analysis

The cytotoxicity was conducted using brine shrimp lethality test[26]. The brine shrimp eggs were placed in 1 L of sea water, aerated for 48 h at 37 °C to hatch become nauplii. After 48 h, ten brine shrimp nauplii were placed in a small container filled with sea water. *A. ilicifolius* flower extract serially diluted with sea water were then added to the container. The lethality of brine shrimp was observed after 24 h of treatment was given. Probity analysis was used to determine lethal concentration (LC_{50}) of *A. ilicifolius* flower methanol extract on nauplii.

2.6. Statistical analysis

Statistical analysis was performed using One-way analysis of variance (ANOVA) and followed by least square difference. Results were expressed as mean±SD from three replications. The P values < 0.01 were considered significant.

3. Results

The phytochemical screening test showed that the flower of *A. ilicifolius* contained several active compounds and dominated polar compounds (Table 1). The antiradical efficiency of *A. ilicifolius* flower extract was presented in Table 2. The percentage scavenging activity of the DPPH by 50% has been used to measure antioxidant activity. A comparable scavenging activity was observed among the

extract of *A. ilicifolius* flower, but the highest antiradical efficiency was obtained from the methanol extract.

Table 1
Phytochemicals of *A. ilicifolius* flower.

Phytochemical	Inference
Triterpenoids	+
Saponin	+
Alkaloids	+
Phenolics	+
Flavonoids	+
Tannins	+
Steroids	–

+: Presence, –: Absence.

Table 2 shows the cytotoxic activity of *A. ilicifolius* flower extracts against brine shrimp nauplii. The cytotoxic activity of extracts of flower *A. ilicifolius* was statistically significant and the strongest activity was exhibited by methanol extract.

Table 2
Antioxidant and cytotoxic activity of *A. ilicifolius* flower extracts (mean ±SD).

Extract	Antioxidant (AE= $\times 10^{-5}$)	Cytotoxic (LC_{50}=μg/mL)
Acetone	6.95 ± 0.15^{b}	1411 ± 27^{b}
Methanol	141.30 ± 2.10^{d}	22 ± 40^{a}
Acetone 70%	12.90 ± 0.90^{c}	2674 ± 66^{c}
Methanol 80%	10.90 ± 0.40^{c}	1348 ± 18^{b}
Water	0.0037 ± 0.0002^{a}	10610 ± 19^{d}

Different letters in the same column indicate significant statistically ($P<0.01$).

4. Discussion

Bandaranayake stated that mangrove is one of active compounds source in the nature[17-19]. *A. ilicifolius* is clustered as true mangrove. The aerial part of *A. ilicifolius* has been extensively investigated for its secondary metabolite content and a large number of compounds have been structurally elucidated[21]. The flower of *A. ilicifolius* inferred positive triterpenoid and this compound in the leaf of this plant have been obtained. For instance lupeol, α amyrin, olcanolic, and ursolic acids[21]. The flower of *A. ilicifolius* contained saponin and triterpenoidal saponin and these compounds have been found in the root of *A. ilicifolius*[27-31]. Tiwara *et al*[32] and Huo *et al*[33] reported that *A. ilicifolius* mangrove contained alkaloids *i.e.* acanthicifoline and benzoxazinium compounds. A wide range of phenolic compounds has been identified in *A. ilicifolius e.g.* acanfolioside, ilicifolioside, acteoside, verbascoside, and apigenin derivatives. Steroids were not screened in our samples while it have been detected in the leaf of *A. ilicifolius* in form of stigmasterol, campesterol, and sitosterol[21].

The methanol extract of *A. ilicifolius* contains phenolic substances clustered as antioxidant compounds[34]. The antioxidant activity of *A. ilicifolius* extract is related to its capability as radical scavenger by transferring proton to free radical, nevertheless the antioxidant capacity of this mangrove species was weaker than ascorbic acid[25]. The capacity of antiradical efficiency of *A. ilicifolius* flower can be classified as medium. The extract was not in pure form; however, it can be categorized as a good and potential antioxidant agent.

The LC_{50} of extracts or pure compounds on brine shrimp or cell line less than 100 μg/mL is categorized as a potential cytotoxic and toxic substance[26,34]. The ethanol leaves extract of *A. ilicifolius* was found to be cytotoxic towards lung fibroblast (L-929) cells in 72 h MTT assay and the concentration required for 50% cell death was 18 μg/mL[23], meanwhile the methanol extract of this plant was cytotoxic to Hela and κB cell line[35]. Wöstmann and Liebezeit[21] reported that this mangrove contained antioxidant substances.

The highest antioxidant and cytotoxic activity were found on methanol extract. Methanol has been known more effective to dissolve active compounds in cells. Hence, it was easier to penetrate the cellular membrane to extract the intracellular ingredients from plant materials. Tiwari *et al.* stated that several active compounds will be obtained if methanol used as solvent in the extraction technique *i.e.* anthocyanins, saponins, tannins, flavones, and polyphenols[36]. These compounds have known as free radical scavenger, reactive species quencher, hydrogen donor, antioxidant enzymes activator, detoxification inducer, normal cell differentiation promoter, tumor production and proliferation cell inhibitor, and apoptosis inducer[23,37-41].

The methanol extract of *A. ilicifolius* flower exhibited both the highest antioxidant and anticancer activities in compare to other extracts. The cytotoxicity of methanol extract can be related to the antioxidant activity and synergism effect of multi-component in extract. Several active compounds contained in the flower of this mangrove have been revealed as a scavenging radical which may be able to inhibit carcinogenesis. Triterpenoid saponin showed its cytotoxicity in HeLa cells through both mitochondrial dysfunction and ER stress cell death pathways, while saponin suppressed tumor invasive and migration by inhibiting MMP-2 and MMP-9 activation[42]. Imai *et al.* determined that flavonoid effectively suppressed the proliferation of a human colon carcinoma cell line, COLO 201, through apoptosis induction while phenolics showed anticancer activity on cancer colon cell by arresting the cell cycle[43-45].

In conclusion, the methanol extract of *A. ilicifolius* flower is potential as antioxidant substances and cytotoxic compounds. Further studies are needed to identify the unknown antioxidant components to establish their pharmacological properties.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

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Comments

Background

The background has demonstrated linkage of the material that will be discussed. It also suggests that a study conducted by the author is still not done by other authors that make it feasible for journal of APJTB.

Research frontiers

Studies on evaluation of active compound from *A. ilicifolius* to cure several diseases have been reported. Hence the extract has not been evaluated on cancer cell toxicity yet. Therefore this article elucidated the best method to extract of antioxidant from the plant that has anti-cancer activity. This finding is novel and will inspire for further research.

Related reports

Potent biological application of *A. illicifolius* was reported. However, in the next the biological properties of *A. illicifolius* should be revealed. From this preliminary report regarding *A. illicifolius* flower can enrich the biological application of mangrove.

Innovations and breakthroughs

This paper showed the best method to extract active compound from *A. ilicifolius* by using methanol. Furthermore, the author also confirmed that the extract has activity as anti-cancer with low dose that warrant for cancer therapeutic.

Applications

The mangrove plant (*A. ilicifolius*) contains a lot of active compounds that have activity as antioxidant. Moreover best method to extract the antioxidant is by using methanol extraction. The magrove also important to remediate costal area, so the conservation of the plant will have mutual benefit for environmental and also human health.

Peer review

The article is explained the best method to extract the active compounds from *A. ilicifolius* that have high antioxidant activity. Moreover the extracts have anti-cancer activity with low dose that warrant cancer therapeutic. This finding is really important for community, scientist and pharmaceutical company as frontier information to explore *A. ilicifolius* as source of anti-cancer material.

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