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Short Communication

Antioxidant Activity and Phenolic Compounds of *Vitex Trifolia* Var, *Simplicifolia* Associated with Anticancer

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Abstract

Vitex trifolia var.simplicifolia plant was found growing wildly at the beach, especially at Pantai Sri Tujuh, Tumpat, Kelantan, Malaysia. The extracted leaves were commonly used to prepare traditional dessert among Siamese communities in Kelantan called "Khanom Bai Kunthi" for a long time. Water was used as an extraction agent by the peoples in this area. This extracted leaves were used by the Siamese communities in Tumpat area because it has natural color, flavor and fragrance properties. The Siamese communities who consumed "Khnom Bai Kunthi" was found to be healthy life and live longer. This is the starting point of the study. For the purpose of this study, Vitex trifolia var.simplicifolia leaves were extracted by using 80% methanol in the laboratory. The extracted V. trifolia var.simplicifolia was analyzed and found to be high in antioxidant activity of phenolic compound. The result shows that the extracted V. trifolia var. simplicifolia had high amount of total phenolic compound (44.66µg of GAE/mg of fresh weight). The plants secondary metabolites normally play role in cancer treatment. Cytotoxicity activity of the extracted V. trifolia var.simplicifolia was evaluated on MCF-7 (Breast cancer cell line), HT-29 (colorectal cancer cell line), and WRL-68 (normal liver cell line), and the results show that, cytotoxicity activity (IC_{50} values 78.87µg/ml, 77.50µg/ml, and 78.29µg/ml, respectively).

Keywords: *Vitex trifolia* var.*simplicifolia*, antioxidant activity, total phenolic content, anticancer activity, cytotoxicity.

Introduction

The utilization of plant based product in food supplements and health industries were increased tremendously for the past four to five years. This was believed to be due to carcinogenic related problems with the usage of artificial or chemicals based products. Therefore, a lot of studies have been done by the researcher all over the world to determine the active bio-component in plant, which could be replaced artificial products. For example, uses *Punica granatum*[1], uses coconut shell and[2] uses *Carica papaya*[3]. Many other researchers were also embarked on the study to identify the possibility of using plant component to solve human health problems[4-7]. Besides that, a lot of studies were also undertaken by the researcher to use plant material as a solution for the environmental pollution, for example,[8] have embarked on a study to utilize Maize Cob to remove heavy metals in industrial wastewater. Meanwhile, study by[9] showed that, *Cassia alata* has a potential to be use as coagulant in water treatment. Similarly with the finding from[10], where they found that, *Moringa oleifera* could be use as natural absorbent and antimicrobial agent in water treatment.

Vitex trifolia var. *simplicifolia* is basically a sea side shrub from the family Lamiaceae or Verbenaceae. The *vitex* genus family is comprised of about 250 species of shrubs and trees; it's widely cultivated in warm temperate and subtropical regions[11] . One of them is *Vitex trifolia* species with variety *simplicifolia.* This species is a creeper wild plant which can be found in sandy soil near beach area. This plant was used to prepare traditional dessert among Siamese communities in Kelantan called "Khanom Bai Kunthi". The ingredients were rice flour, salt and extract of *Vitex trifolia* var. *simplicifolia* leaf. "Khanom Bai Kunthi" is served with grated coconut and granulated sugar. Extracted leaves of *Vitex trifolia* var. *simplicifolia* will give color, flavor and fragrance to the dessert. Color, flavor and fragrance properties were believed to be from the pigments in the leaf.

The main factor which determined the color of the tissues is plant's pigments such as chlorophyll, xantophyll, carotene, flavone, flavonol, and anthocyanin. Chlorophyll pigments possessed by *V. trifolia* var. *simplicifolia* allow photosynthesis process to occur at the cellular level, allowing plants to appear green[12]. Chlorophyll is not a very stable compound, thus it can be destroyed due to certain temperature and pH changes[13]. However, as chlorophyll destroyed, the other pigments such as carotenoid and anthocyanin are expressed[12].

In other case, the color of the anthocyanin can be influenced by pH[14]. The anthocyanin turns into bright pink in acidic solution, reddish-purple in neutral solutions and green in alkaline or basic solutions[15]. Anthocyanins are antioxidant flavonoids which improved human health condition. Besides, antioxidant supplementation can block NF-kB (nuclear factor kappa-light-

chain-enhancer of activated B cells) activation as well as inhibit NF-kB activity. Since NF-kB is responsible to cancer and inflammation, thus it indirectly plays important role to inhibit cancer and inflammantion through mechanisms distinct from redox regulation[16]. Physiological and biochemical processes in human body may produce oxygen-centered free radicals and other reactive oxygen species as byproducts. Overproduction of such free radicals can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, including cancer[17].

Figure -1
Vitex trifolia-simplicifoli

The present study has been undertaken to screen the active compounds in *Vitex trifolia* var. *simplicifolia* leaf, other than natural color, flavor and fragrance properties to relate the finding with the good health and longevity of peoples who consumed "Khanom Bai Kunthi".

Material and Methods

Sample Collection: *V. trifolia* var. *simplicifolia* samples were collected from Pantai Seri Tujuh, Kelantan, Malaysia.

Sample Extraction: The leaves samples were blended in 80% methanol. Then, the solvent was removed from samples using rotary evaporator/rotavap (Büchi, Switzerland). Freeze dryer was used to transform the samples into powder form.

Total Phenolic Content (TPC) Assayed: The total phenol content was assayed according to the Folin-Ciocalteu method[18]. Test samples were diluted to a concentration of 1 mg/ml in methanol. Test solution (0.05 mL of 1 mg/mL solution) was added to 3 mL of distilled water and swirled before added with 0.25 mL of Folin-Ciocalteu phenol reagent. After 2 min, 0.75mL of 20% sodium carbonate was added and the volume made up to 5 mL with distilled water. The mixture was vortexed and left for 2 h, after which the absorbance was measured at 760nm. A mixture without test solution was used as blank. 1 mg/mL solution of gallic acid was used as standard, and the total phenolic content of each extract was expressed as percentage of the phenolic content of gallic acid (taken as 100%). Spectrophotometer measurements were performed at least three times for each separate concentration of standard and samples, and in triplicate.

Cytotoxicity assays: The cytotoxicity activities of test samples were performed against MCF-7 (Breast cancer cell line), HT-29 (colorectal cancer cell line), and WRL-68 (normal liver cell line). Briefly, cells in 100 µl of medium per well were seeded in 96-well flat-bottomed micro titer plate. After 24 hours of incubation in a 5 % CO_2 humidified incubator at 37^{O}C, 100 µl of media containing test samples were added into micro filter plate in quadruplicate at various concentrations. After 72 hours incubation, the cells were fixed by adding 50 µl of TCA for 30 min at RT, rinsed with tap water and stained with 0.4% SRB (in 1%acetic acid) to remove unbound dye, air-dried and solubilised in 100 µl of 10 mM unbuffered tris base solution. The plates were read in micro-plate reader at 490 nm. Results were expressed as the dose that inhibited 50% control growth after the incubation period (IC_{50}). The values were estimated by plotting drug concentration (µg/ml) against the percentage of viable cells compared to control. The tests were repeated in at least three independent experiments.

Results and Discussion

The extracts of *V. trifolia* var. *simplicifolia* give rise to purple liquid. The use of the 'kunthi' leaves as natural food products have more advantages compared to other product which contained synthetic/artificial coloring, fragrance and flavorant. Natural food product can provide beneficial values such as antioxidant and anticancer that synthetic product do not provide. Furthermore, FDA (food and drug administration) also has firmer rules on the use of synthetic coloring such as tartrazine and amaranth for food coloring agent.

Analysis of the extract shows high antioxidant activities of phenol contents with 44.66µg of GAE/mg (44.66 mg GAE/g) of fresh weight. The high level activities of antioxidant in 'kunthi' leaves give major advantage for medicinal purposes. NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls the transcription of DNA. NF-kB plays a key role in regulating the immune response to infection. Conversely, incorrect regulation of NF-kB has been linked to cancer.

The nuclear factor (NF-kB) proposed to be a pivotal protein in the link between inflammation and cancer[19]. NF-kB characterizes all inflammatory responses and is also a major hallmark of tumors[20]. Antioxidant supplementation can block NF-kB activation and inhibit NF-kB activity through mechanisms distinct from redox regulation[16]. Meanwhile, cytotoxicity activity of the extracted *V. trifolia* var.*simplicifolia* which was tested on breast cancer cell line, colorectal cancer cell line and normal liver cell line shows that, cytotoxicity activity, IC_{50} values 78.87µg/ml, 77.50µg/ml, and 78.29µg/ml, respectively. Cytotoxicity test of 'kunthi' leaves against cancer cell line have proved to inhibit cancer cell line which is one of the major causes of human death.

Table-1
Cytotoxicity of *V.trifolia var. simplicifolia* against cancer cell lines

Cell Lines	IC_{50} (µg/ml)
MCF-7 (Breast cancer cell line)	78.81
HT-29 (Colorectal cancer cell line)	77.50
WRL-68 (Normal liver cell line)	78.29

Conclusion

The significance findings of this study was that the *V. trifolia* var. *simplicifolia* demonstrated much stronger antioxidant activity and contained significantly more phenolics (44.66 mg GAE/g) than common vegetables and fruits which are considered as good natural sources of dietary antioxidants. Based on literature reviews, red apple contain 11.6 mg GAE/ g^{21}, sour prickly pears contain 2.07 mg GAE/g^{22} , red pitaya contains 0.424 mg GAE/ g^{23} and white mulberry contain 19.24 mg GAE/g^{24}. The *V. trifolia* var. *simplicifolia* plants might be a potential source of excellent natural antioxidants and anticancer to be use also as natural color, flavour and fragrance for food preparation. Phenolic antioxidants in the *V. trifolia* var. *simplicifolia* plants and their antioxidative properties would play a role in preventing and treating cancer.

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