Health promoting Potential of Selaginella and Mistletoe Decoction: Antioxidant and Cytotoxic Activities

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Abstract

The aim of this study was to observe the effect of combination of selaginella and mistletoe aqueous extract on DPPH free radical scavenging activity and in vitro cytotoxic activity on MCF-7 cells. Total phenolic and flavonoid content of extracts were measured by Folin-Ciocalteau and aluminium chloride colorimetric assay respectively. The DPPH scavenging assay was used to measure antioxidant activity. Then, MTT assay was used for in vitro cytotoxic activity. Results showed that antioxidant activity of mistletoe aqueous extract was higher than selaginella aqueous extract. Antioxidant activity of selaginella aqueous extracts was increased by addition of mistletoe aqueous extracts compared to its single treatment. The total phenolic and flavonoid content of mistletoe aqueous extract were higher than selaginella aqueous extract.

The results of cytotoxic activity showed that selaginella and mistletoe aqueous extracts inhibited MCF-7 cells growth and its combination showed synergistic effect (CI<1). The combination of selaginella and mistletoe aqueous extracts exhibited synergistic effect on both antioxidant and cytotoxic activity and potential to be developed for health supplement.

Keywords: Selaginella, mistletoe, DPPH, cytotoxic activity, health supplement.

Introduction

Natural product showed its preventive activity for several diseases[25]. Food and beverages are examples of natural product consumed on daily life. Nowadays, natural product that have addition function for health promotion are studied[20,24]. According to these facts, selaginella and mistletoe also have been studied for its bioactivities. Selaginella is used as one kind of raw or fresh vegetables in Indonesia[22]. Selaginella is traditionally used to cure several diseases such as wound, postpartum, menstrual disorder, skin disease, headache, fever, infection, cirrhosis, cancer, rheumatism and bone fracture[21].

Ethanolic extract of *Selaginella plana* and its active fraction inhibits cancer proliferation and induces apoptosis on T47D and MCF-7 cells[10,11,19]. Ethyl acetate fraction from selaginella water infusion shows anti-diabetes and cytotoxic activity[3]. On the other hand, water decoction of mistletoe is traditionally used for increasing of body health. Studies of bioactivities of mistletoe prove that mistletoe has relationship with improving of immune system, antioxidant, antidiabetes and cytotoxicity[1,4,8,16]. Then, selaginella and mistletoe are promising candidates for health supplement.

Decoction is one of extraction methods by boiling plant materials with water. This method becomes an easy way to drink herbal supplements. Nevertheless, the bioactivity study of selaginella and mistletoe decoction especially in combination study is limited. Then, this study observed the effect of combination of selaginella and mistletoe decoction (aqueous extract) on DPPH free radical scavenging and *in vitro* cytotoxic activity.

Material and Methods

Preparation of extracts: Selaginella (*Selaginella sp.*) and avocado mistletoe (*Scurrulla sp.*) raw material were cleaned, dried and followed with powder. Extract decoction was prepared using dried powder of each plant and water solvent (1:10) and built for 1 h. Then, water decoction was separated from the solid and was dried. The extracts (aqueous extract) thus obtained were used directly to assess its bioactivities.

Total phenolic content: The amount of total phenolics content was measured by Folin-Ciocalteau method[26] with slight modifications. Initially, 20 µL of diluted extracts or standard were added to 96 well-plates and followed with 140 µL of dH_2O. Furthermore, 10 µL of Folin-Ciocalteau reagent (Merck) was added and the mixture was gently shaken for 5 minutes. Then, 30 µL of Na_2CO_3 (20%, w/v) were added, gently shaken and incubated for 2 h in the dark. The absorbance was measured using a microplate reader (Varioskan Flash, Thermo) at 750 nm. The results were expressed as % total phenolic content [mg gallic acid (Sigma-Aldrich) equivalent/g extract].

Total flavonoid content: The amount of total flavonoid content was measured by aluminium chloride colorimetric assay [6,17] with slight modifications. Initially, 20 µL of diluted extracts or standard were added to 96 well-plates and followed with 88 µL of dH_2O. Then 6 µL of $NaNO_2$ (5%, w/v) were added and incubated for 5 minutes. Then, 6 µL of AlCl3 (10%, w/v) were added and the mixture was gently shaken for 6 minutes in the dark. Furthermore, 80 µL of 1M NaOH was added, the mixture was gently shaken and absorbance was measured using a microplate reader (Varioskan Flash, Thermo) at 420 nm. The results were expressed as % total flavonoid content [mg quercetin (Sigma-Aldrich) equivalent /g extract].

DPPH radical scavenging activity: Radical scavenging activity was measured by DPPH radical scavenging method, described by Artanti et al[2] with slight modifications. 160 µL of various concentrations of extracts in single and combination were added to 96 well-plates. 40 µL of 1 mmol/L methanolic solution of DPPH was added, gently shaken and incubated for 30 min at RT. The absorbance was measured using a microplate reader (Varioskan Flash, Thermo) at 515 nm.

Cell culture: MCF-7 cell line was kindly provided by Prof. Edy Meiyanto (CCRC, UGM, Indonesia) and was cultured in CO_2 incubator (37°C) with Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA) with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific), 0.5% amphotericin B (Thermo Fisher Scientific) and 1.5% penicillin-streptomycin (Thermo Fisher Scientific).

Cytotoxic assay: The cells (1×10^4/well) in 96-well plates were treated with various concentrations of the different treatment groups for 24-h incubation. Cells were then incubated for 4 h with 100 µL of culture medium and 10 µL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich) in each well. The MTT reaction was stopped using dimethyl sulfoxide (DMSO) (Sigma-Aldrich). The absorbance was measured with a microplate reader (Varioskan Flash, Thermo Fisher Scientific) at 595 nm.

Statistical analysis: The combination index (CI) was calculated using CI equation developed by Raynold and Maurer[18].

Results and Discussion

Total phenolic and flavonoid content: Selaginella and mistletoe, rich of phenolic and flavonoid have been reported[7,12]. Nevertheless, its contents in aqueous extract might be less than in organic solvent because of the dissolution ability of the compounds. The total phenolic contents of selaginella and mistletoe aqueous extracts were 46 ±3.2 gallic acid equivalents/g and 160.1 ±5.0 gallic acid equivalents/g respectively. Then, the total flavonoid content was 129 ±6.1 quercetin equivalents/g and 253±19.3 quercetin equivalents/g respectively (Table 1).

The results showed that total phenolic and flavonoid contents of mistletoe aqueous extract were higher than selaginella aqueous extract. Phenolic and flavonoid compounds have a role on antioxidant mechanism. Then, we determined antioxidant activity of selaginella and mistletoe aqueous extracts.

Antioxidant activity: Herbal decoction formula generally consists of multiple ingredients[15]. Here, we were supposed to observe antioxidant activity of selaginella and mistletoe aqueous extracts in single and combination using DPPH free radical scavenging assay.

The results showed that single treatment of selaginella and mistletoe aqueous extracts increased DPPH free radical scavenging activity in a dose-dependent manner (Fig. 1A-B) with IC_{50} value of 604 ± 28.8 µg/ml and 13 ± 0.1 µg/ml respectively (Fig. 1D). Nevertheless, the activity of both of the extracts was less than quercetin (IC_{50} = 6 ± 1.4 µg/ml) (Fig. 1C-D). The DPPH free radical scavenging activity of mistletoe aqueous extract was higher than selaginella aqueous extract.

Furthermore, we observed combination treatment of selaginella and mistletoe aqueous extracts with series of concentrations to confirm whether these combination enhanced DPPH free radical scavenging activity compared to its single treatment. The synergistic antioxidant activity of the combination was measured by combination index (CI) parameter.

The results showed that DPPH free radical scavenging activity of selaginella aqueous extracts was increased by addition of mistletoe aqueous extracts compared to its single treatment (Fig. 2A). Combination of selaginella and mistletoe aqueous extracts showed CI values less than 1 (Fig. 2B). The combination treatment showed that combination of selaginella and mistletoe aqueous extracts exhibited synergistic antioxidant activity.

Cytotoxic activity: In order to verify the cytotoxic activity of selaginella and mistletoe aqueous extracts, cells were treated with both extracts in single and in combination using MTT assay. The results showed that selaginella and mistletoe aqueous extracts inhibited MCF-7 cells growth in a dose-dependent manner (Fig. 3A-B) with IC_{50} value of 2.6 ± 0.18 mg/ml and 4.7 ± 0.3 mg/ml respectively (Fig. 3D).

These results showed that the cytotoxic activity of selaginella and mistletoe aqueous extracts was less than quercetin (IC_{50} = 0.75 ± 0.04 mg/ml) (Fig. 3C). However, these compounds are potential to be developed as chemoprevention agent.

Table 1
Total phenolic and flavonoid content of selaginella and mistletoe aqueous extracts.

S.N.	Aqueous extract	Total phenolic content (mg/g GA)	Total flavonoid content (mg/g Qe)
1.	Selaginella	46 ±3.2	129 ±6.1
2.	Mistletoe	160.1 ±5.0	253±19.3

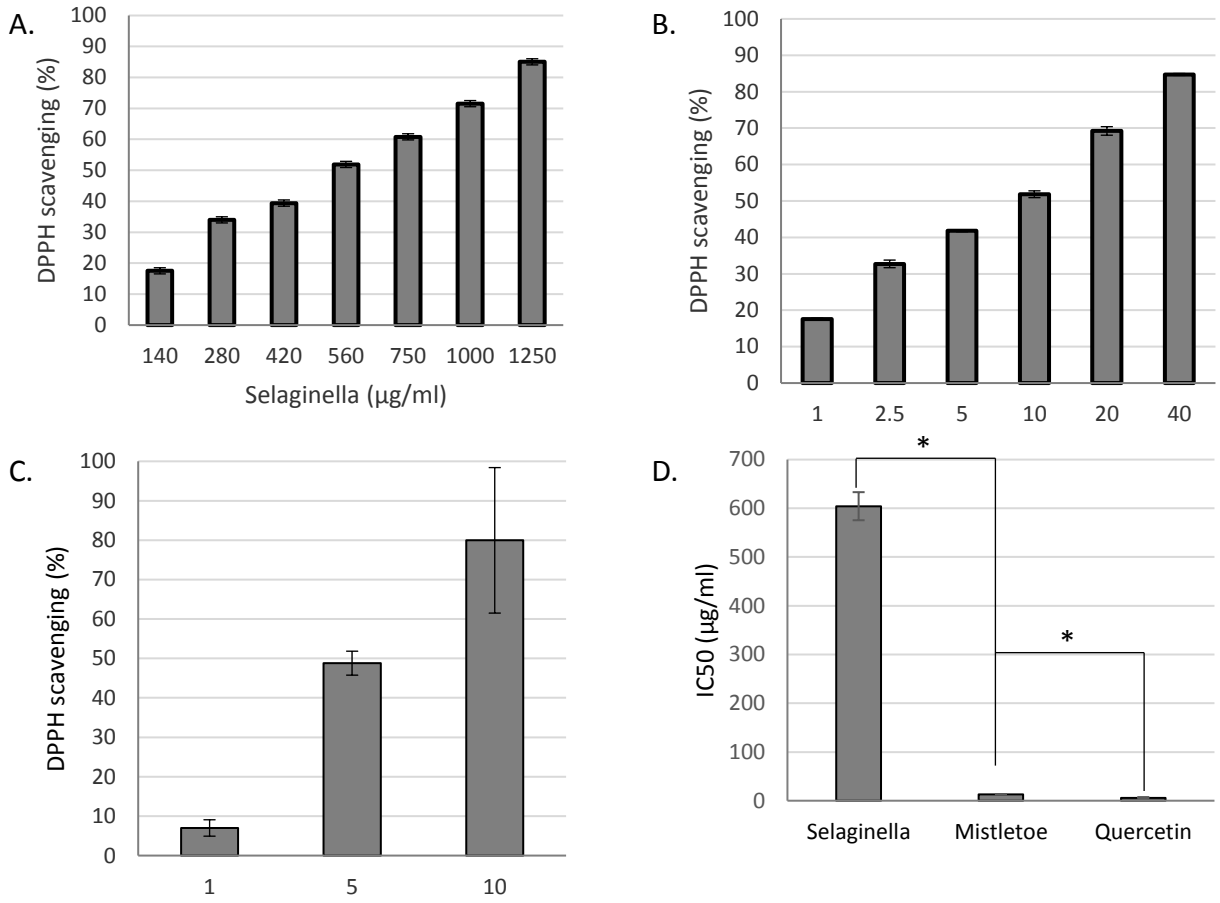

Figure 1: Antioxidant activity of single treatment of selaginella and mistletoe aqueous extract using DPPH free radical scavenging assay. Single treatment of (A) Selaginella, (B) Mistletoe, (C) Quercetin and (D) its IC_{50} values were calculated according to the method. Data represent the mean ± SD (n = 3).

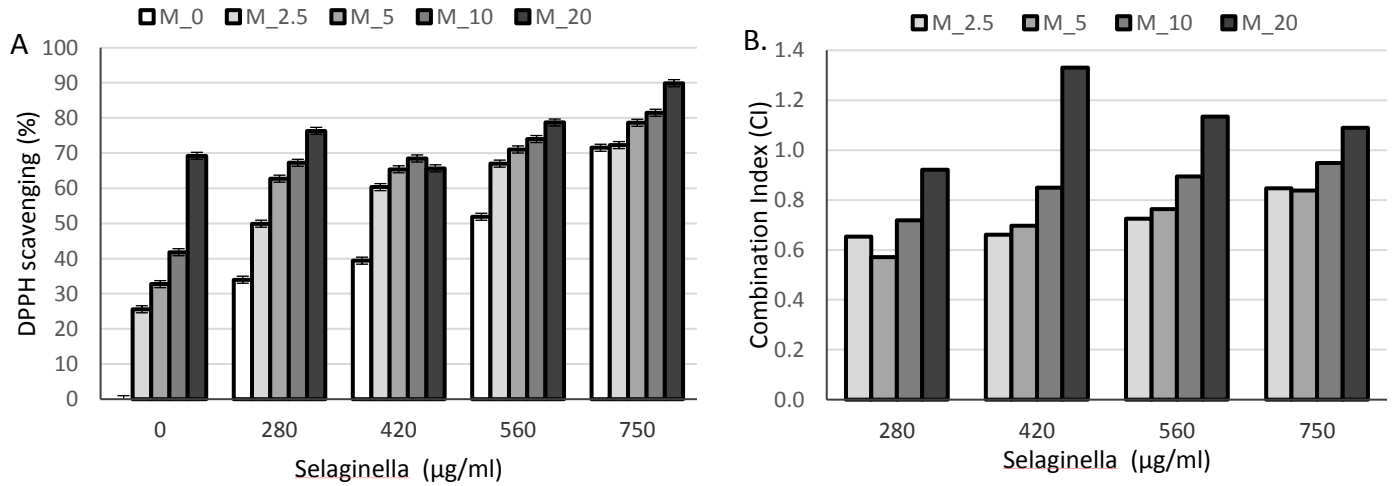

Figure 2: Antioxidant activity of combination of Selaginella and Mistletoe (M) aqueous extracts using DPPH free radical scavenging assay. (A) Antioxidant activity and (B) its CI value were calculated according to the method. Error bar represents standard deviation (n = 3).

Next, we analyzed combination treatment of Selaginella and Mistletoe aqueous extracts with series of concentrations to confirm whether these combination enhanced cytotoxic activity compared to its single treatment. The synergistic cytotoxicity activity of the combination was measured by combination index (CI) parameter. The results showed that cytotoxic activities of combination of Selaginella and Mistletoe aqueous extracts on MCF-7 cells were higher than its single treatment (Fig. 4A). Combination of Selaginella and Mistletoe aqueous extracts showed CI values less than 1

(Fig. 4B). The combination treatment showed that combination of selaginella and mistletoe aqueous extracts exhibited synergistic effect on inhibition of MCF-7 cells growth.

Total phenolic and flavonoid contents of mistletoe were higher than selaginella (Table 1). This result was in line with its antioxidant activity (Fig. 1A-B). The redox properties in phenolic and flavonoid compounds allow them to act as antioxidants. The free radical scavenging ability of phenolic and flavonoid compounds facilitated by their hydroxyl groups can be used for rapid screening of antioxidant activity[23]. On the contrary, even though selaginella and mistletoe aqueous extracts had cytotoxic effect against MCF-7 cells, the cytotoxic effect of selaginella was higher than mistletoe. This result showed that compound with high antioxidant activity was not always gives the great cytotoxic effect compared to compound with less antioxidant effect.

Artanti et al[2] reported that fraction of *Syzygium cumini* that showed the best antioxidant effect did not show the best cytotoxic activity. The similar result was shown on the comparison of antioxidant and anticancer effect of trihydroxyflavones. This study reported that antioxidant and anticancer effect of trihydroxyflavone only showed a moderate relationship[9]. Antioxidant has a relation mechanism with down-regulation of reactive oxygen species (ROS). Even though cancer cells with overexpression of ROS need antioxidant in order to escape from cells death, the ROS–mediated signaling pathways activate pro-oncogenic signaling which has a role in cancer progression, angiogenesis and survival[13].

We suggested that Selaginella has an important role on cytotoxic activity of cancer cells and antioxidant effect of mistletoe may act to boost this activity. Cytotoxic effect of single treatment of selaginella has been reported[10,11,14,19]. As an addition, antioxidant effect of mistletoe may have a benefit on this combination through its protection from normal cells damage. Azqueta and Collin [5] reported that low concentrations of antioxidant compound tend to protect cells damage. Nevertheless, the further study needs to be done.

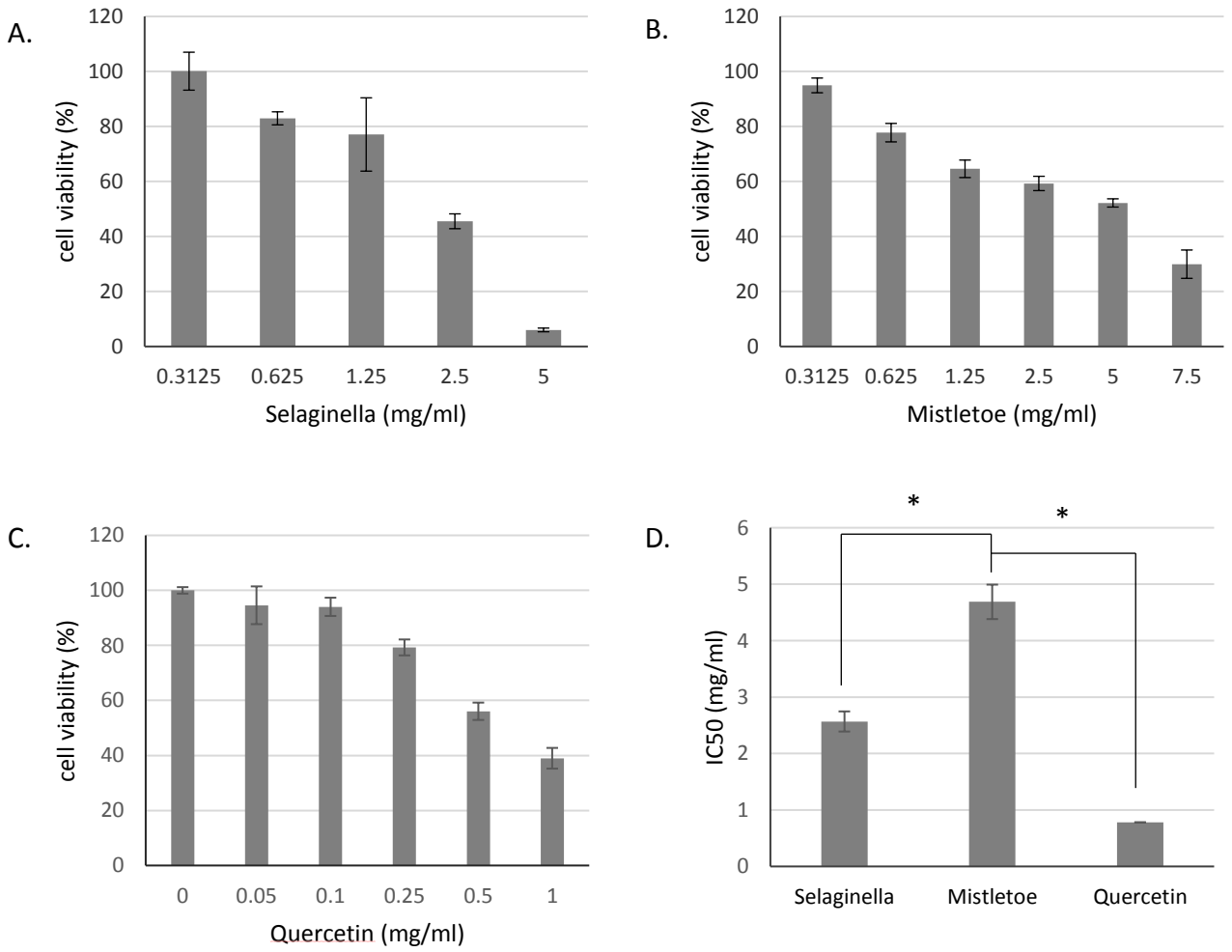

Figure 3: Cytotoxic activity of single treatment of Selaginella and Mistletoe aqueous extracts on MCF-7 cells. Cytotoxic effect of (A) Selaginella aqueous extract, (B) Mistletoe aqueous extract, (C) quercetin and (D) its IC_{50} values on viability of MCF-7 cells. Various concentrations of samples were added to the cells for 24 h before assessment by MTT assay. Error bar represents standard deviation (n = 3, *$P < 0.05$ by Student's *t* test).

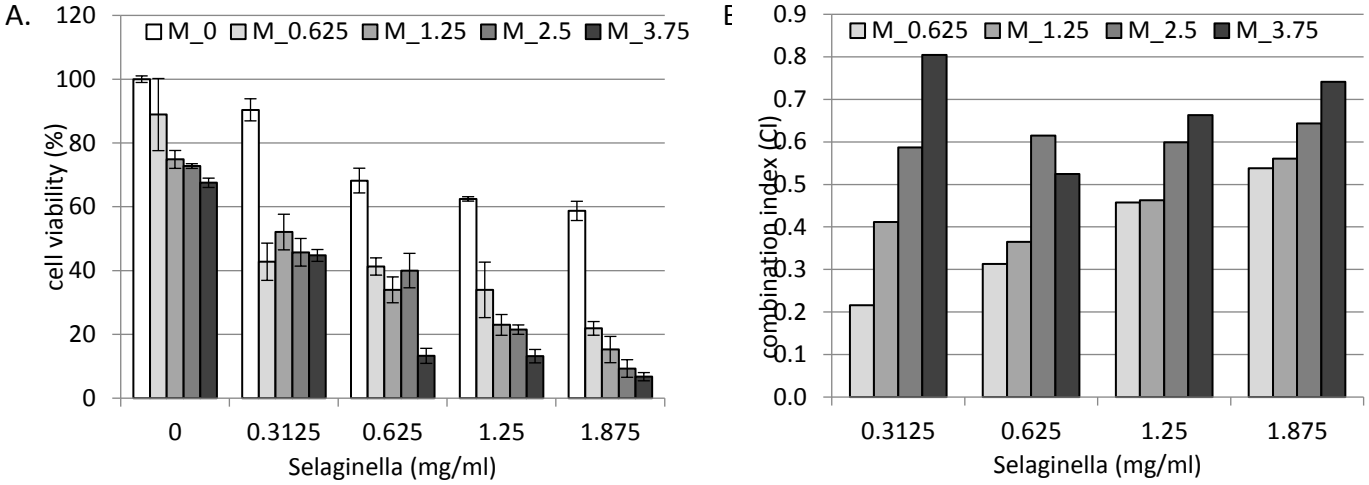

Figure 4: Cytotoxic activity of combination of Selaginella and Mistletoe (M) aqueous extracts using MTT assay. (A) Cytotoxic activity and (B) its CI value were calculated according to the method. Error bar represents standard deviation (n = 3).

Conclusion

Combination of Selaginella and Mistletoe has antioxidant and cytotoxic activities. Then, this combination has potential to be developed for health supplement.

Acknowledgement

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