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Original Article

Evaluation of antibacterial, antioxidant activity and *Calmodulin* gene expression of *Scoparia dulcis* Linn.

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Abstract

In Thailand, *Scoparia dulcis* is being used as a traditional medicine. The extract displays the best inhibitory effect against *Pseudomonas aeruginosa* and its minimum inhibitory concentration (MIC) was 400 µg/mL followed by *Escherichia coli* and *Staphylococcus aureus* with MIC 500 µg/mL. The presence of the extract significantly protected *Sf*9 cells against H_2O_2-induced cell death. In addition, the extract also showed DNA damage inhibitory effect in a concentration-dependent manner (0.5-4 mg/mL). However, at higher concentrations (7.5-30 mg/mL) it might induce damage to the DNA. The prevention of DNA damage differs in different parts of the plant. To support secondary metabolite synthesis in different parts of *S. dulcis*, we investigated the expression of the *Calmodulin* gene that is involved in secondary metabolite production. The *Calmodulin* gene showed the highest expression in the fruit. This finding justifies the use of *S. dulcis* in the treatment of diseases caused by bacteria and free radicals.

Keywords: *Scoparia dulcis* Linn., antibacterial, H_2O_2, DNA nicking, *Calmodulin* gene expression

1. Introduction

The exposure of organisms to various infections has been reported to generate a wide range of reactive oxygen species (ROS) which results in hundreds of disorders especially in diabetic wound infection (Ivanov, Bartosch, & Isaguliants, 2017). Traditional medicinal plants are being used as a strategy for the production of effective novel compounds to develop new antimicrobial and antioxidant drugs. Medicinal plants contain a wide variety of therapeutic agents with biochemical and therapeutic properties.

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Scoparia dulcis (SD) Linn. (Plantaginaceae), commonly known as sweet broom, is a herbal medical plant widely used in the indigenous system of medicine. *S. dulcis* is found in tropical and subtropical regions. Fresh or dried *S. dulcis* has been used to treat liver ailments, stomach troubles, ulcer, cancer, wounds, cough, and tuberculosis. A previous report revealed that an aqueous crude extract from *S. dulcis* effectively protected rodents from gastric ulcers (Mesia-Vela *et al.*, 2007). The SD extract and its active compounds may exert effects in cases of diabetes and as an anticancer agent (Beh, Latip, Abdullah, Ismail, & Hamid, 2010; Wu, Chen, Lu, Chen, & Chang, 2012). In addition, SD extract was reported to promote wound healing and showed good blood-clotting abi-lity in the rat model (Ediriweera, Jayakody, & Ratnasooriya, 2011; Krishna, Vijay, Mayank, & Megha,

2011). These biological activities are conceivably attributed to its active phytochemical constituents. *S. dulcis* accumulates a variety of secondary metabolites such as scoparic acid A, scoparic acid B, scopadulcic acid A, scopadulcic acid B, scopadulciol, sco-padulin, scopanolal, and scopadiol with a group of saponins (Pamunuwa, Karunaratne, & Waisundara, 2016). Nkembo *et al.* showed that the synthesis of secondary metabolites was enhanced when *S. dulcis* was stimulated by methyl jasmonate and calcium. The *Calmodulin* gene functions as the key com-ponent in the activation of the calcium cascade in these pro-cesses. The production of scopadulcic acid B (SDB) was almost completely blocked by pretreatments with trifleopera-zine (Calmodulin antagonists) (Nkembo, Kurosaki, Lee, & Hayashi, 2005; Saitoh *et al.*, 2007). Plant *Calmodulin* is in-volved in plant responses to environmental stimuli and stresses, such as salinity, cold, heat, oxidative stress, and pa-thogens. In tobacco, *Calmodulin* gene expression was en-hanced in response to a pathogenic *Ralstonia solanacearum* infection (Takabatake *et al.*, 2007). Transgenic soybean ex-pressing a foreign *Calmodulin* gene showed pathogen stress tolerance (Rao *et al.*, 2014).

Hence, the present study was aimed to provide scientific data on the beneficial biological properties including antibacterial and antioxidant properties of *S. dulcis*. In addition, gene expression analysis of *Calmodulin* was performed in different plant tissues. This investigation suggests that the function of *Calmodulin* may be related to the secondary metabolite production of *S. dulcis* which exerts potent antimicrobial effects and protection of cells and DNA damage from free radicals. The consequence of this investigation supports that *S. dulcis* can be used for the treatment of microbial infections in wounds especially in diabetic wounds.

2. Materials and Methods

2.1 Collection and identification of plant samples

Plant samples were collected from a local area in Songkhla Province, Thailand. The subject plant material specimen (N. Chana 001) was authenticated by a taxonomist, Assistant Professor Dr. Jarun Leerativong, Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla, Thailand. The specimen was deposited at the herbarium of Prince of Songkla University, Thailand. The fresh young leaves were grounded into powder in liquid nitrogen and used for the genomic DNA extraction. Genomic DNA isolation was performed using a GeneJET Plant Genomic DNA Purification Kit (Thermo Scientific) according to the manufacturer's instructions. The ITS2 region was amplified using the following pair of universal primers: ITS-2F (5′-GTT TCC GTA GGT GAA CCT-3′) and ITS-2R (5′-GCT TAT TAA TAT GCT TAA ATC ACG-3′). The PCR products were cloned into pGEM-T easy vector and sent for DNA sequencing.

2.2 Preparation of *S. dulcis* extract

The aerial parts of the plant were collected and 200 grams of fresh plant were soaked in 300 mL of methanol for 3 days with occasional shaking. The resultant mixture was then successively filtered through Whatman filter paper No 1. A rotary evaporator was used to concentrate the filtrate at 40 °C. These extracts were then used for a biological properties study. The concentrated sample was dissolved in dimethyl sulfoxide (DMSO)/Mueller-Hinton broth (MHB) to carry out the antibacterial activity. In a separate experiment, the root, leaves, stem, and fruit of *S. dulcis* were extracted according to the method described above and dissolved in distilled water for DNA nicking assay.

2.3 Preparation of inoculums

The four strains of bacteria used in this study included Gram-positive bacteria (*Staphylococcus aureus* AT CC25923, methicillin-resistant *Staphylococcus aureus* SK1), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC27 853, and *Escherichia coli* ATCC25922). The bacteria were subcultured on nutrient agar (NA). Five single colonies were transferred into nutrient broth (NB) and incubated in a shaking incubator at 35 ºC, 150 rpm for 3 h. McFarland standard No. 0.5 was used to adjust the turbidity of bacterial suspension. The suspension was then diluted to a 1:200 ratio with MHB.

2.4 Antibacterial assay

The antibacterial activity of the crude extract was evaluated with the colorimetric broth microdilution method. The extract which exhibited the antibacterial activity at 1 mg/mL was further subjected to the same method to find the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC).

2.4.1 Preliminary assay

Antibacterial activity tests were conducted using a 96-well microtiter plate by the colorimetric broth microdilution method (Clinical and Laboratory Standards Institute [CLSI], 2012) with some modifications. Fifty-micro liters of crude extract (2 mg/mL) and 50 µL of bacterial suspension (10^6 CFU/mL) were added in each well of the microtiter plate according to the method of Supaphon *et al*. (2013) Plates were incubated at 35 ºC for 18 h. Then, 10 µl of resazurin indicator was added to the plates. The results were recorded as growth or no growth based on the color of the resazurin (blue color) as a positive result and resorufin (pink color) as a negative result (Supaphon, Phongpaichit, Rukachaisirikul, & Sakayaroj, 2013).

2.4.2 Determination of MIC and MBC

The MIC was determined by the previous assay using a 2-flow dilution of crude extract with final concentrations of 1000-100 µg/mL. The lowest concentration of extract that inhibited growth of microorganisms was recorded as the MIC value. All extracts in the microtiter plate that showed a blue color were further tested to find the minimum bactericidal concentration. The blue color in each well was streaked onto NA. Plates were incubated at 35 ºC for 18-24 h. The lowest concentration of extract that showed no growth of bacteria on NA was recorded as the MBC value. Antibiotic drugs (vancomycin and gentamicin) were used as positive controls and DMSO was used as the negative control.

2.5 *Spodoptera frugiperda* (*Sf9*) insect cell viability assay

Sf9 insect cells (Invitrogen) were cultured in insect express Sf9-S2 medium. The cells were seeded onto a 96-well plate (5×10^4 cells/well) and grown to approximately 70-90% confluence for 24 h. The cells were incubated with SD extract (2 mg/mL or 4 mg/mL), and the mixture was also combined with 750 µM or 1 mM H_2O_2 diluted in the Sf9 culture medium for 16 h. Viability of the cells was assessed using the colorimetric microtiter (MTT) survival assay. The percentage of cell survival was calculated and compared with untreated cells.

2.6 DNA nicking assay

To study the ability of SD extract to protect supercoiled DNA from metal-catalyzed oxidation (MCO), *in vitro* tests were performed essentially as described by Saelee *et al.* with slight modifications (Saelee, Tonganunt-Srithaworn, Wanna, & Phongdara, 2012). Approximately 200 ng of pUC19 plasmid incubated with different concentrations of the extract followed by addition of 10 mM dithiothreitol and 3 µM of $FeCl_3$. The negative control was without addition of the extract. The reaction was then incubated 2 h at 37 °C. The samples were then analyzed by 0.8% agarose gel electrophoresis.

2.7 Tissue distribution analysis of *Calmodulin*

To determine the gene related to secondary metabolite production, the expression level of *Sd-Calmodulin* was analyzed using the RT-PCR technique. The leaves, stem, fruit, and root were detached from *S. dulcis*. Total RNA of *S. dulcis* was extracted using the GeneJET RNA Purification Kit according to the manufacturer's instructions (Thermo Fisher Scientific). The single-stranded cDNA was synthesized using SuperScript® III Reverse Transcriptase (RT) and oligo (dT) primer, following the manufacturer's protocol (Invitrogen). The transcript level of *Sd-Calmodulin* gene was amplified using the following primer pair: CAM-SdF (5'-GAG GCT GAG CTT CAG GAT ATG ATC AA) as the forward primer and CAM-SdR (5'-ACG TCG GCC TCG CGG ATC ATC TCG T-3') as the reverse primer. Actin was used as the internal control with specific primers: Actin-SdF (5'-TCT ACA ATG AGC TCC GTG TTG C-3') and Actin-SdR (5'-CTT GTT CAT AAT CAA GAG CAA TGT A-3'). The amplified DNA fragments were subcloned into pGEM-T Easy vector (Promega Corporation), and sent for DNA sequencing.

2.8 Guava ViaCount assay

A Guava ViaCount assay was used with the Guava easyCyte 5HT Flow cytometer to evaluate viable and dead cell populations on Chinese hamster ovary (CHO) cells exposed to SD extract. The cells were exposed to the SD extract for 24 h. Guava ViaCount Reagent was added to the cells. The percentage of viable cells was assessed by flow cytometry and analyzed with Guava ViaCount software. The results were expressed as the percentage of live cells in treated cultures compared to those present in the control cultures.

2.9 Guava Nexin® assay

A Nexin assay was used to evaluate viable, early and late apoptotic cell populations on CHO cells exposed to SD extract. CHO cells were exposed to the extract for 24 h. The cells were stained with Guava Nexin Reagent and data analysis was performed using the Nexin software module.

3. Results and Discussion

3.1 Identification of *S. dulcis*

In order to facilitate accurate species resolution when testing the herbal products, we tested the nuclear ribosomal internal transcribed spacer 2 (ITS2) gene in identification of herbal medicine species. ITS is one of the most sequenced regions in molecular systematics. The ITS2 sequences were analyzed using the BLAST method in the GenBank database. DNA analysis showed that *S. dulcis* ITS2 sequence from Songkhla Province in Thailand has 99% identity with *Scoparia dulcis* species (Accession number AY963776.1). The *S. dulcis* is a member of the Gratioleae tribe in the Plantaginaceae family. Our results showed that the ITS2 gene could successfully identify this species of herbal medicine.

3.2 Antibacterial property

Bacterial infections caused by drug-resistant bacteria are at an increased risk in Thailand. Natural products are alternative sources of new pharmaceutical agents, especially Thai medicinal plants. *S. dulcis* is a flowering plant distributed in southern Thailand. It has been widely reported that there are various phytochemical compounds in whole plants such as flavonoids, saponins, tannins, alkaloids, steroids, and terpenes (Wankhar, Srinivasana, & Rathinasamy, 2015). The extracts of this plant species were used to treat diarrhea, ulcer, and wounds (Ediriweera *et al.*, 2011). Wounds with bacterial infection are the most common public health problems. Most studies of *S. dulcis* used dried plant material to extract the metabolites and tested their ability against pathogenic microorganisms (Jose, & Sinha, 2017; Latha *et al.*, 2006; Zulfiker *et al.*, 2011; Mohandas, Valsalakumari, William, & Narayanan, 2014). There is no report on the antibacterial activity of extract from fresh plant material. We first investigated the effect of methanolic extract from the fresh aerial part of *S. dulcis* (SD extract) on the antibacterial activity. SD extract was found to inhibit both bacterial strains (Gram-positive and Gram-negative bacteria). The extract was most active against *P. aeruginosa* with MIC 400 µg/mL, followed by *E. coli* and *S. aureus* with MIC 500 µg/mL (Table 1).

Coulibaly *et al.* (2012) revealed that dried *S. dulcis* extract exhibited significant antimicrobial activities, methanolic extract and aqueous-acetone extract on *E. coli* (MIC 12.5 mg/mL), aqueous-acetone extract on *S. typhimurium* (MIC 1.56 mg/mL), chloroform extract on *B. cereus* (MIC 1.56 mg/mL). Furthermore, hexane extract from this plant displayed the best antifungal activity against *A. niger* and *P. roquefortii* with MIC 6.25 mg/mL (Coulibaly *et al.*, 2012). The result in this study indicated that fresh SD extract yielded more antibacterial activity than dried SD extract as previously

Table 1. Potential of SD extract and its antibacterial activity against four bacterial strains at MIC and MBC concentrations ranging from 100 to 1000 µg/mL.

Samples	MIC/MBC (µg/mL)			
	E. coli[1]	*P. aeruginosa*[2]	*S. aureus*[3]	MRSA[4]
SD extracts	500/>1000	400/>1000	500/>1000	>1000/>1000
Gentamicin	0.5/2	0.25/2		
Vancomycin			0.25/0.25	0.25/0.25

MIC=minimum inhibitory concentration, MBC=minimum bactericidal concentration. [1]*Escherichia coli* ATCC25922, [2]*Pseudomonas aeruginosa* ATCC27853, [3]*Staphylococcus aureus* ATCC25923, and [4]methicillin-resistant *Staphylococcus aureus* SK1.

reported. It could also be suggested that the extraction components in fresh and dried SD extract might have a different composition. SD extract was also reported to have activities against the viruses such as herpes simplex virus type 1, pathogenic fungi and *Aedes aegypti* larvae (Hayashi, Masaru, Yoshihisa, Tooru, & Naokata, 1990; Wankhar *et al.*, 2015). These findings confirm that S. *dulcis* is a good source of active metabolites.

3.3 Protective effects of SD extract against H_2O_2 damage in *Sf9* cells

Wound infections are a common problem for the diabetic population. ROS such as superoxide anion ($\cdot O2^-$), hydroxyl radicals ($\cdot OH$), and hydrogen peroxide (H_2O_2) play an integral role in the wound healing process particularly the hydrogen peroxide radical. However, excessive ROS can damage the structure of nearby molecules via an oxidation reaction. The antioxidants were designed to remove the deleterious effects of ROS in wound infection (Dunnill *et al.*, 2015). Natural plant products have been a useful source of antioxidant properties that may act as free radical scavengers to prevent oxidative stress imbalance. SD extract was reported to exhibit various pharmacological activities, including wound healing, anti-diabetic, anti-inflammatory, and anti-oxidative effects (Ediriweera *et al.*, 2011; Latha, Pari, Sitasawad, & Bhonde, 2004; Murti, Panchal, Taya, Singh, 2012). The present study focused on the ability of SD extract to protect *Sf9* cells against H_2O_2 exposure. Cell viability was measured by a MTT assay. The MTT assay revealed that exposure of *Sf9* cells to H_2O_2 at 750 µM and 1 mM, significantly decreased cell viability (Figure 1). However, treatment of *Sf9* cells with fresh SD extract increased cell viability. These results suggest that SD extract exerts a cytoprotective effect against H_2O_2-induced oxidative stress in *Sf9* cell. Latha *et al.* showed that *S. dulcis* extract also prevented streptozotocin-induced rat insulinoma cell death (Latha *et al.*, 2004). The aqueous extract of *S. dulcis* plays a role in hepatoprotective and antioxidant activity against N-nitrosodiethylamine induced hepatotoxicity in experimental rats (Langeswaran, Jagadeesan, & Vijayaprakash, 2012). Thus, SD extract has a cytoprotective role against cytotoxic agents.

In the future, the SD extract will be applied to mammalian cells. Therefore, in this study, we also used CHO cells instead of *Sf9* cells to evaluate cell viability and cytotoxicity of the SD extract. We used Guava ViaCount flow cytometry assays for the detection of cell viability. After a 24-h treatment of CHO cells with 2 mg/mL of SD extract, the percentage of viable cells was 97% and at 4 mg/mL cell viability was 93.2% (Figure 2). Further analyses were carried out for an apoptosis assay in CHO cells by the Guava Nexin® assay. The results indicated an increase in the percentage of apoptotic cells at 4 mg/mL of SD extract in comparison with control cells (Figure 3). Hence, 2 mg/mL of SD extract will be used to test the biological activity in CHO cells in the future.

3.4 DNA protection analysis

ROS are able to destroy macromolecules, such as DNA, proteins, and lipids in cells. The DNA damage is central to the development of diseases such as cancers and degenerative diseases (Jackson & Bartek, 2009). The present study

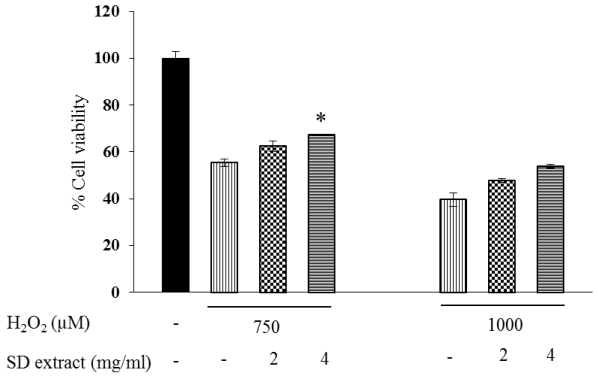


Figure 1. The percentage of *Sf9* viable cells after incubation with or without different concentrations of SD extract and treatment with or without H_2O_2. The viable cells were monitored using MTT assay. *P<0.05 compared with H_2O_2 treated cells. Data are expressed as mean±SD (n=3).

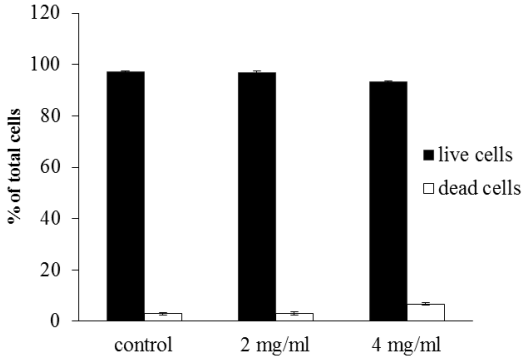


Figure 2. Evaluation of the effect of SD extracton cell viability by ViaCount assay in Chinese hamster ovary (CHO) cells. Cell populations obtained by Guava ViaCount flow cytometry after 24-h incubation of CHO cells with SD extract at 2 mg/mL or 4 mg/mL. Results are expressed as percentage (%) of live or dead cells. Each bar represents mean±SD of three independent experiments.

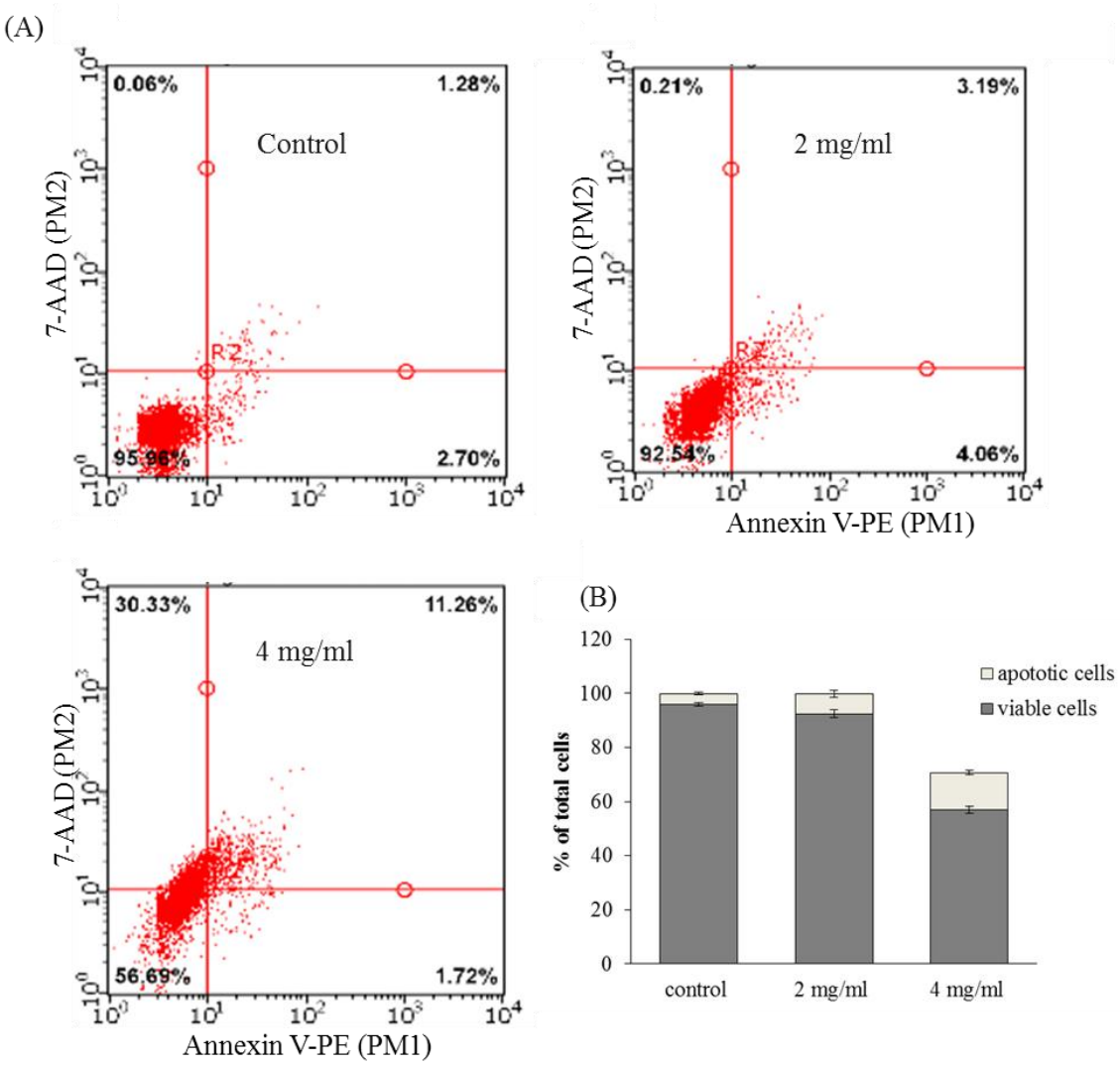
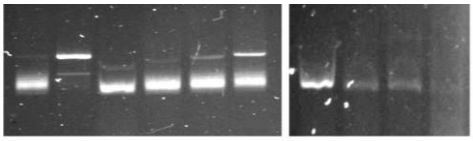


Figure 3. Cell apoptosis was evaluated by Guava Nexin® assay following 24-h incubation of Chinese hamster ovary cells with or without 2 mg/mL or 4 mg/mL of SD extract. (A) Data represent dot plot of apoptosis analysis. (B) Results expressed as mean percentage (%) of viable and apoptotic cells. Results are expressed as mean±SD of three independent experiments.

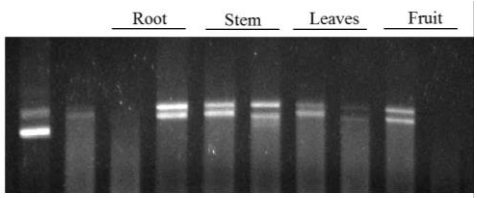
aimed to evaluate DNA damage protection activity of SD extract *in vitro*, to provide scientific basis for traditional usage of this plant. An *in vitro* DNA nicking assay was used to study the potential of antioxidant substances in the plant extract (Leba *et al.*, 2014). The pUC 19 plasmid DNA without incubation with MCO components did not bring about any damage to supercoiled DNA (Figure 4, lane 1), whereas complete nicking of supercoiled DNA occurred in the MCO system without SD extract (Figure 4, lane 2). The aerial part of the SD extract prevented oxidative damage of the supercoiled DNA in the presence of a DNA damaging agent in a dose-dependent manner at a concentration of 0.5-4 mg/mL (lanes 3-6) (Figure 4). The results suggested that SD extract possesses supercoiled DNA protection activity against MCO. However, at high concentrations (7.5-30 mg/ mL) it might induce reductive damage to the DNA structure (lanes 7-10) (Figure 4). Plant polyphenol compounds are active antioxidants, which can transfer electrons to radical species to protect the DNA from radicals (Jackson *et al.*, 2009; Nimse & Pal, 2015). However, excessive antioxidants might cause these compounds to transfer their electrons to nucleotide bases and induce radical species which induce chemical bond cleavage in DNA (Lu, Ou, & Lu, 2013). This study showed similar results with fermented red brown rice extract in a DNA nicking analysis (Kong, Lee, Michelle, Ginjom, & Nissom, 2015). These activities of SD extract may be attributed to the presence of various secondary metabolites such as flavonoids and phenolic acids which were reported earlier to neutralize the radicals. Hence, the secondary metabolite accumulation in different tissues of *S. dulcis* may result in different DNA damage protection levels.



Lane	1	2	3	4	5	6	7	8	9	10
pUC19	+	+	+	+	+	+	+	+	+	+
DTT	-	+	+	+	+	+	+	+	+	+
$FeCl_3$	-	+	+	+	+	+	+	+	+	+
Aerial part SD extract (mg/ml)	-	-	4	2	1	0.5	7.5	15	25	30

Figure 4. DNA protection assay of SD extract from the aerial part of the plant. Treatment of pUC19 plasmid with SD extract in absence and presence of metal-catalyzed oxidation followed by 0.8% agarose gel electrophoresis analysis.

The leaves, stem, fruit, and root were detached from *S. dulcis* to study the DNA damage protection. The results showed that the radicals produced in the MCO system caused complete degradation of the control DNA within 2 h of incubation (Figure 5, lane 2). The root part of SD inhibited DNA damage at 4 mg/mL (Figure 5, lane 4). However, at the concentration of 0.5 mg/mL it did not show protective action against DNA damage (Figure 5, lane 3). The stem extract prevented DNA damage at concentrations of 0.5 and 4 mg/mL (Figure 5, lanes 5-6). Extract of the leaves showed DNA damage protection at 0.5 mg/mL (Figure 5, lane 7), but at a concentration of 4 mg/mL it may induce about 50% DNA damage (Figure 5, lane 8). The protective effects of fruit extract against DNA damage were shown at a concentration of 0.5 mg/mL (Figure 5, lane 9). However, it completely damaged the DNA at a concentration of 4 mg/mL (Figure 5, lane 10). Thus, the prevention of DNA damage depends not only on concentration but also on the origin of the extract. SD extract from the stem part was most effective in preventing DNA damage. However, only the fruit extract completely damaged the DNA at a concentration of 4 mg/mL. These results may imply that the fruit part may have the highest secondary metabolite content because it can induce reductive damage to the DNA structure. However, further chemical composition level analysis in SD extract is necessary to confirm this suggestion.



Lane	1	2	3	4	5	6	7	8	9	10
pUC19	+	+	+	+	+	+	+	+	+	+
DTT	-	+	+	+	+	+	+	+	+	+
$FeCl_3$	-	+	+	+	+	+	+	+	+	+
Different tissues of SD extract (mg/ml)	-	-	0.5	4	0.5	4	0.5	4	0.5	4

Figure 5. Protection of supercoiled DNA cleavage by SD extract from different tissues. Treatment of pUC19 plasmid with SD extract in absence and presence of metal-catalyzed oxidation followed by 0.8% agarose gel electrophoresis analysis.

3.5 Tissue distribution analysis of the *Calmodulin* gene

Various parts of *S. dulcis* are used extensively in traditional medicine to cure various human ailments and its extract has antibacterial and antioxidant properties. However, the mechanism is still unknown. It has been documented that *Calmodulin* gene expression increased in ROS treatment in the protoplasts of mesophyll cells. This mechanism enhanced the expression of antioxidant genes such as *superoxide dismutase 4* (*SOD4*), *cytosolic corbate peroxidase* (*cAPX*), and *glutathione reductase 1* (*GR1*). The up-regulation of the antioxidant enzymes was almost completely blocked by pretreatments with Calmodulin antagonists (Hu *et al.*, 2007). Moreover, Nkembo *et al.* (2005) revealed that the production of scopadulcic acid B (SDB) was almost completely blocked by pretreatments with trifluoperazine (Calmodulin antagonists) (Nkembo *et al.*, 2005; Saitoh *et al.*, 2007). SDB was found to possess various biological activities, such as an inhibitor against replication of herpes simplex virus type 1, gastric H^+, K^+-ATPase (Riel, Kyle, & Milhous, 2002) and antitumor promoting activity (Nishino, Hayashi, Arisawa, Satomi, & Iwashima, 1993). The DNA nicking assay in the present study revealed that the fruit extract may have higher secondary metabolite content in comparison to other plant parts. To support the secondary metabolite synthesis in different parts of the *S. dulcis*, the *Calmodulin* gene participating in secondary metabolite production was isolated and its gene expression was investigated in different organs using reverse transcription polymerase chain reaction. *Sd-Calmodulin* showed the highest expression in fruit and this may result in higher concentrations of secondary compounds in the fruit compared to other tissues (Figure 6).

In conclusion, the SD extract was found to inhibit both Gram-positive and Gram-negative bacteria, and protected *Sf*9 cells against H_2O_2-induced oxidative damage. The extract also prevented DNA damage from MCO. These activities of SD extract may be attributed to the presence of various secondary metabolites. The expression of the *Calmodulin* gene involved in secondary metabolite production is highest in the fruit. Our data suggested that *S. dulcis* should be considered as a useful source of material for the treatment of wound infection as an antioxidant and antimicrobial agent.

Acknowledgements

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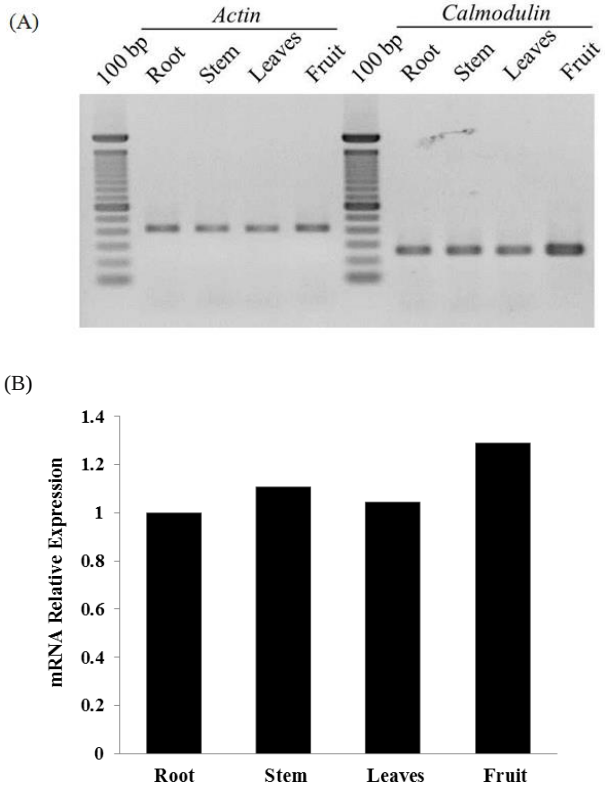

Figure 6. Transcriptional activity of *Sd-calmodulin* was determined by RT-PCR. (A) PCR products were analyzed by 1.5% agarose gel electrophoresis. (B) Image analysis with Quantity One software from Bio-Rad.

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