DEVELOPMENT OF *AZIMA SARMENTOSA* TWIGS SOAP ON ANTIOXIDANT CAPACITY AND CHEMICAL COMPOSITION

Sasipen Krutchangthong[1], Kitthisak Khlaeo Chansukh[2], Rumrada Meeboonya[3] and Chawalit Yongram[4]

[1,2]Division of Applied Thai Traditional Medicine, College of Allied Health Sciences, Suan Sunandha Rajabhat University;

[3,4]Division of Cannabis Health Sciences, College of Allied Health Sciences, Suan Sunandha Rajabhat University;

E-mail: sasipen.kr@ssru.ac.th[1], kitthisak.ch@ssru.ac.th (K.C.)[2], rumrada.me@ssru.ac.th (R.M.)[3]; chawalit.yo@ssru.ac.th (C.Y.)[4]

**Corresponding author, E-mail: sasipen.kr@ssru.ac.th*

ABSTRACT

Soap was produced thousands of years ago by reacting animal fats and ashes obtained from plants which used for personal hygiene and laundry. Natural soap is one alternative soap, made from natural substances, such as essential oil or plant extract. The *A. sarmentosa* was reported for antioxidant and anti-inflammatory activities. In this study, *A. sarmentosa* twigs soap was produced in 4 formulations which are 0%, 1%, 2.5% and 5% w/w of extract and antioxidant capacity and chemical composition were analyzed. The results showed that the *A. sarmentosa* twigs soap has a good antioxidant capacity in a concentration-dependent manner. Moreover, the TPC, TFC and TCC showed the higher chemical content than CT, soap without extract. For physical properties, our results showed physical properties of *A. sarmentosa* twigs soap were within the criteria and showed the physical properties higher than CT. As a result, the findings indicate that *A. sarmentosa* twigs soap has the potential for antioxidant activity and soap production for skincare products.

Keywords: *Azima sarmentosa*, Soap, Antioxidant capacity, Chemical composition, Physical properties

INTRODUCTION

The sensory and chemical characteristics of natural soaps are dependent on the manufacturing process, and the chemical composition of the feedstock materials used during formulation. For example, the type and purity of base (alkali) used determines the hardness and solubility of the finished soap. Sodium hydroxide produces harder, more durable soaps, while potassium hydroxide is used to produce soft soap bars or liquid soaps (Vivian, et al.,2014) However, the natural soaps are generally defined as alkali salts of fatty acids derived primarily from vegetable or plant oils used as soap feedstock, and contained natural fragrances and/or organic ingredients included as additives. Commercially, natural soaps are manufactured via either a cold or hot saponification process, where triglycerides in fats, oils,and/or free fatty acids used as feedstock are converted in the presence of a base (typically sodium or potassium hydroxide) to form fatty acid salts (soaps), glycerol, and free fatty acids (Friedman, et al,.1996).

Medicinal plants that have been found in saline soil areas include *Gisekia phanaceoides*, *Maytenus mekongensis*, *Synostema bacciformis*, *Pluchea indica*, and *Azima*

sarmentosa (Sankla et al., 2021). The *Azima sarmentosa* (Blume) Benth. & Hook. F. is medicinal plant belonging to the genus *Azima*, family Salvadoraceae. The family Salvadoraceae consists of three genera such as *Dobera*, *Salvadora* and *Azima* (Ronse De Craene and Wanntorp, 2009). Moreover, the genus *Azima* showed antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective activities (Sankla et al., 2022; Kekuda and Raghavendra, 2017). *A. sarmentosa* was distributed in Hainan, Southeast Asia, New Guinea, Philippines and Thailand (Sankla et al., 2022).

Two main groups of chemical compounds can be used as anti-aging which are the antioxidant agent and the cell regulators. Antioxidant agents are compounds that come from plants, such as vitamins, polyphenols and flavonoids can reduce collagen degradation by decreasing the concentration of free radicals in the tissues (Ganceviciene et al.,2012).

OBJECTIVES

3.1 To determine the antioxidant capacity of *A. sarmentosa* twigs soap

3.2 To analyze the chemical composition and physical properties of *A. sarmentosa* twigs soap

MATERIALS AND METHODS

2.1. Sample preparation

2.1.1 Plant material

Mature leaves and stems of *Azima sarmentosa* (Blume) Benth. & Hook. f. were collected from Samut Songkhram, Thailand in January 2024. Voucher specimens of *A. sarmentosa* (S. Krutchangthong 001) were deposited at Division of Cannabis Health Sciences, College of Allied Health Sciences, Suan Sunandha Rajabhat University (SSRU 002).

2.1.2 Extraction

The *A. sarmentosa* twigs were cut and mashed to dry powder. The twigs powder (338.02 g) was macerated with 95%ethanol 400 mL and extracted sonification by ultra sonicator (GT sonic®, Guangdong, China) for 30 min at room temperature and repeated three times. After that, extract was filtered with Whatman No.1 filter paper and evaporate to dry the filtrate by using rotary evaporator to obtain *A. sarmentosa* twigs ethanolic extract. Then, weigh the extract and keep it for future use.

2.1.3 Soap production

Soap production was prepared for 4 formulations which are 0%, 1%, 2.5% and 5% w/w of twigs ethanolic extract. There are ingredients as described in Table 1, weighing 10 g per spoon. Briefly, glycerin base soap was heated to solution. After that, the extract was added to glycerin base soap solution and mixed. Then, the soap solution was added into the soap molding size 2.9 cm x 2.9 cm x 1.5 cm.

Table 1. The ingredients of *A. sarmentosa* twig soap

Soap formulations	Concentration of extract (%W/W)	Glycerin soap base (g)	Twig extract (g)
CT	0.00	10.00	0.00
T1.0	1.00	9.90	0.10
T2.5	2.50	9.75	0.25
T5.0	5.00	9.50	0.50

2.2 Antioxidant capacities

2.2.1 DPPH radical scavenging method

The 0.2 mM DPPH solution was mixed with sample and incubated for 30 min at room temperature. Then, the mixture was measured at 517 nm by a microplate reader (EZ Read 2000, Biochrom, USA). The DPPH value was calculated in milligrams of Trolox equivalent per gram of soap (mg TE/g soap) (Yongram et al., 2022).

2.3 ABTS radical scavenging method

The 7 mM $ABTS^{+}$ solution was mixed with sample and incubated for 10 min at room temperature. Then, the mixture was measured at 735 nm by a microplate reader. The ABTS value was calculated in milligrams of Trolox equivalent per gram of soap (mg TE/g soap) (Sripan et al., 2022).

2.4 Chemical contents

2.4.1 Total phenolic content (TPC)

Total phenolic content was analyzed by Folin-Ciocalteu assay. The sample was mixed with the 10%Folin-Ciocalteu into 96-well plate. After that, 7%Sodium carbonate (Na_2CO_3) and incubated for 30 minutes at room temperature. Then, the mixture was measured at 760 nm by a microplate reader. The result was calculated in milligrams of gallic acid equivalents per gram of soap unit (mg GAE/g soap) (Panyatip et al., 2022).

2.4.2 Total flavonoid content (TFC)

Total flavonoid content was analyzed by Aluminum chloride colorimetric assay. The sample was mixed with the 2%Aluminum chloride ($AlCl_3$) into 96-well plate and incubated for 20 minutes at room temperature. Then, the mixture was measured at 415 nm by a microplate reader. The result was calculated in milligrams of quercetin equivalents per gram of soap unit (mg QE/g soap) (Yongram et al., 2022).

2.4.3 Total chlorophyll content (TCC)

The sample (10 mg/mL in MeOH) 200 µL was added into 96-well plate. The absorbance was measured at 645 and 663 nm by microplate reader. Total chlorophyll content was calculated by equation (1) in milligrams per gram soap unit (mg/g soap) (Arnon, 1949; Tamprasit et al., 2019).

$$\text{Total chlorophyll content (mg/g soap)} = [(20.2 \times A_{645}) + (8.02 \times A_{663})] / (1000 \times W) \quad (1)$$

where W is sample weight (g)

2.5 Physical properties analysis

2.5.1 Foreign matters

The foreign matters of *A. sarmentosa* twig soap was observed under a stereo microscope with 10x zoom magnification (Phansi et al., 2023).

2.5.2 pH

A. sarmentosa twig soap 1 g was dissolved in distilled water 100 mL. Then, pH value was measured by a pH mete (TOA-DKK, Tokyo, Japan) (Phansi et al., 2023).

2.5.3 Foaming volume and foaming stability

A. sarmentosa twig soap 1 g was dissolved in distilled water 20 mL and transferred into a cylinder size 100 mL. After that, the soap solution was shaked for 40 times and recorded the Foaming volume at 0 min and 5 min. Then, the foaming volume and foaming stability were calculated by equation (2) and (3), respectively (Phansi et al., 2023).

$$\text{Foaming volume (mL)} = V_{\text{at 0 min}} - V_{\text{distilled water}} \quad (2)$$

$$\text{Foaming stability (mL)} = V_{\text{at 5 min}} - V_{\text{distilled water}} \quad (3)$$

where V is volume of foam (g)

2.4.4 %Erosion

A. sarmentosa twig soap 10 g was added into distilled water 500 mL at 40°C for 1 min. The soap was rotated in the palm area for 40 times. After that, soap was washed with distilled water and repeated for 3 times. Then, the soap was weighed and the %erosion was calculated by equation (4) (Phansi et al., 2023).

$$\%\text{Erosion} = [(W_{\text{before}} - W_{\text{after}}) / W_{\text{before}}] \times 100 \quad (4)$$

where W is sample weight (g)

2.6 Statistical analysis

The experiments were performed in three replicates and the results are expressed as means ± standard deviation (SD). The One-way ANOVA analysis was used for comparing the sample and control group using Tukey's HSD (Tukey's Honest Significant Difference) test at $p<0.05$ by SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

3.1 Extraction and soap production

The *A. sarmentosa* twigs 338.02 g was macerated with 95%ethanol 400 mL to obtain the *A. sarmentosa* twigs ethanolic extract 13.79 g or 4.08% yield. Then, the *A. sarmentosa* twigs ethanolic extract was used to produce the soap three formulations such as twig 1%, twig 2.5% and twig 5% and CT as soap without extract in Figure 1.

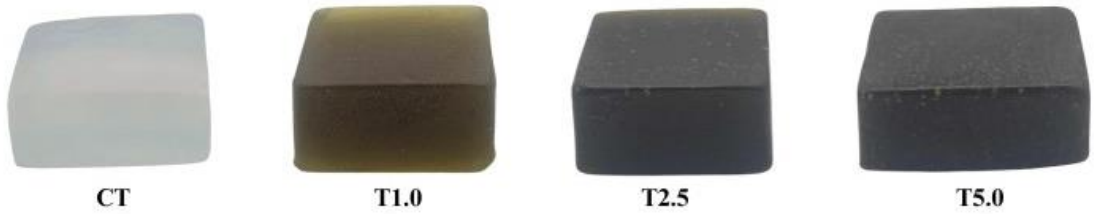

Figure 1. The three formulations *A. sarmentosa* soap; twigs 1%, twig 2.5% and twig 5% and CT as soap without extract

3.2 Antioxidant capacity

The *A. sarmentosa* twigs soap showed a good antioxidant capacity in Table 2. In DPPH assay, the result showed the DPPH value of T1.0, T2.5 and T5.0 formulations with values of 0.03±0.00, 0.06±0.00 and 0.13±0.00, mg TE/g soap, respectively. ABTS assay present antioxidant capacity of T1.0, T2.5 and T5.0 formulations with values of 0.17±0.01, 0.26±0.00 and 0.38±0.01 mg TE/g soap, respectively. The T5.0 formulation have a significantly high antioxidant capacity in DPPH and ABTS assays with values of 0.13±0.00 and 0.38±0.01 mg TE/g soap, respectively which is higher than CT, soap without extract.

Table 2. Antioxidant capacity of *A. sarmentosa* twigs soap

Soap formulations	DPPH value (mg TE/g soap)	ABTS value (mg TE/g soap)
CT	ND^{d}	0.26±0.02^{b}
T1.0	0.03±0.00^{c}	0.17±0.01^{c}
T2.5	0.06±0.00^{b}	0.26±0.00^{b}
T5.0	0.13±0.00^{a}	0.38±0.01^{a}

Note: The different letters indicated the significance between rows in the same column. ND is not detected.

3.3 Chemical composition

The *A. sarmentosa* twigs soap showed the total phenolic content in range 1.48 to 1.87 mg GAE/g soap which is higher than CT, significantly (Table 3). Moreover, total flavonoid content showed the value of T1.0, T2.5 and T5.0 formulations with TFC values of 3.81±0.15, 4.52±0.20 and 4.90±0.19 mg QE/g soap. However, only T2.5 and T5.0 formulations showed TFC higher than CT, significantly. For chlorophyll analysis, the TCC of all formulations present the total chlorophyll content lower than CT.

Table 3. Chemical composition of *A. sarmentosa* twigs soap

Soap formulations	TPC (mg GAE/g soap)	TFC (mg QE/g soap)	TCC (mg/g soap)
CT	1.20±0.05^{c}	4.14±0.12^{b,c}	0.08±0.03^{a}
T1.0	1.48±0.05^{b}	3.81±0.15^{c}	0.00±0.00^{b}
T2.5	1.49±0.06^{b}	4.52±0.20^{a,b}	0.01±0.00^{b}

Soap formulations	TPC (mg GAE/g soap)	TFC (mg QE/g soap)	TCC (mg/g soap)
CT	1.20±0.05^{c}	4.14±0.12^{b,c}	0.08±0.03^{a}
T5.0	1.87±0.02^{a}	4.90±0.19^{a}	0.04±0.01^{a,b}

Note: The different letters indicated the significance between rows in the same column.

3.4 Physical properties

A. sarmentosa twigs soap physical properties which are foreign matters, pH value, foaming volume, foaming stability and erosion were analyzed (Table 4). The result showed that the under stereo microscope with 10x zoom magnification not found foreign matters in *A. sarmentosa* twigs soap and CT formulation. The *A. sarmentosa* twigs soap showed pH value in range of 9.42-9.50 and CT have pH value of 9.36. For foaming volume, the *A. sarmentosa* twigs soap showed the foaming volume of T1.0, T2.5 and T5.0 formulations with value of 23.33±0.94, 28.67±0.94 and 30.67±0.94 mL, respectively, which T2.5 and T5.0 formulations have foaming volume than CT, significantly. Moreover, *A. sarmentosa* twigs soap demonstrated the foaming stability value the significantly higher than CT, which T5.0 have the highest foaming stability with value of 36.33±2.87 mL. In addition, CT showed the highest %erosion with value of 12.25±0.70, while three *A. sarmentosa* twigs soap sowed a %erosion in the range 14.42-16.09%.

Table 4. Physical properties of *A. sarmentosa* twigs soap

Soap formulations	Foreign matters	pH value	Foaming volume (mL)	Foaming stability (mL)	%Erosion
CT	Non	9.36±0.00^{d}	21.00±1.63^{b}	11.00±0.82^{d}	12.25±0.70^{b}
T1.0	Non	9.50±0.01^{a}	23.33±0.94^{b}	19.00±0.82^{c}	14.42±0.91^{a}
T2.5	Non	9.46±0.00^{b}	28.67±0.94^{a}	26.67±1.25^{b}	14.98±0.56^{a}
T5.0	Non	9.42±0.00^{c}	30.67±0.94^{a}	36.33±2.87^{a}	16.09±0.13^{a}

Note: The different letters indicated the significance between rows in the same column.

DISCUSSIONS

The *A. sarmentosa* twigs soap showed a good antioxidant capacity in rang 0.03-0.13 mg TE/g soap by DPPH assay and ABTS assay showed value in rang 0.17-0.38 mg TE/g soap. The 3 formulations of *A. sarmentosa* twigs soap has antioxidant capacity higher than CT. In addition, the *A. sarmentosa* twigs soap demonstrated the antioxidant capacity in concentration-dependent manner. The results indicated that the *A. sarmentosa* twigs have potential for antioxidant activity. Moreover, *A. sarmentosa* stem was reported the antioxidant activity with IC_{50} value of 232.80 µg/mL and anti-inflammatory activity (Sankla et al., 2022). The accumulation of ROS was related to pathogenic factors in the aging process of the skin. The ROS acts to upregulate the expression of both elastase and tyrosinase enzymes, which subsequently leads to wrinkle formation, lack of elasticity, and hyperpigmentation (Chaikhong et a., 2023). The antioxidant agents can support potential therapies by reducing reactive oxygen species (ROS) levels (Kaźmierczak-Barańska et al., 2020).

Chemical composition of *A. sarmentosa* twigs soap demonstrated the moderate chemical content which is TPC value of 1.48 to 1.87 mg GAE/g soap, TFC value of 3.81 to 4.90 mg QE/g soap and TCC value of 0.00 to 0.04 mg/g soap. Our result shows that the *A. sarmentosa* twigs soap has the chemical composition in concentration-dependent manner. The chemical composition of *A. sarmentosa* was reported the alkaloids, phenolic, flavonoids, coumarin, tannins and terpenoids were present in the stem extract (Sankla et al., 2022). These compounds are secondary metabolites from medicinal plants with beneficial pharmacological effects (Wu et al., 2023). Moreover, the total phenolic content related to antioxidants in plants (Yongram et al., 2019) and flavonoid contents remarkable scavenging radical (Phuyal et al., 2020). In addition, chlorophylls can act as antioxidant activity (Pérez-Gálvez et al., 2020).

For physical properties, the criteria of soap follow a pH value of 8-10, no foreign matters, enough foaming volume, good foaming stability and less minimal erosion (Thitiwongsawet et al., 2016). Our results showed physical properties of soap were within the criteria. However, the *A. sarmentosa* twigs soap showed lower foaming volume and foaming stability than soap from mulberry leaf tea, while showing less erosion than soap from mulberry leaf tea (Phansi et al., 2023).

CONCLUSION

In conclusion, *A. sarmentosa* twigs soap presents an antioxidant capacity higher than soap without the extract (CT), significantly. which is T5.0 formulation showed the highest antioxidant capacity with DPPH and ABTS assays in a concentration manner. In addition, the *A. sarmentosa* twigs soap showed a high chemical composition such as TPC, TFC and TCC. Moreover, physical properties analysis demonstrated no foreign matters in all soap formulations. The foaming volume and foaming stability of *A. sarmentosa* twigs soap were significantly higher than CT. However, *A. sarmentosa* twigs soap has %erosion more than CT, significantly. Therefore, the result suggests that A. *sarmentosa* twigs have the potential for antioxidant activity and the development of soap for skin products.

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