RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2021.068

ScienceAsia 47 (2021): **549–555**

Evaluation of anti-inflammatory, cytotoxic, anti-*H. pylori*, antioxidant activities, and phytochemical compositions of *Shirakiopsis indica* (Willd.) Esser

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Received 10 Jan 2021
Accepted 4 Jun 2021

ABSTRACT: *Shirakiopsis indica* (Willd.) Esser (Sa-Mor-Ta-Lay, in Thai), a herbal plant belonging to the family Euphorbiaceae, has traditionally been used to treat gastrointestinal symptoms. The objectives of this study were to investigate the bioactivities (cytotoxic, antioxidant, anti-inflammatory, and anti-*Helicobacter pylori*) of *S. indica* extracts that influence the treatment of gastric inflammation. The chemical constituents of the extracts were also analyzed by performing LC-ESI-QTOF-MS/MS; and they are tentatively identified as Sapintoxin A, Sapintoxin B, Sapintoxin C, Saipinin, and a few other phorbol esters. We found that the ethanolic extract of *S. indica* exhibited the highest cytotoxicity against the Kato III gastric cancer cell line and the highest inhibition of nitric oxide production with the IC_{50} values of 3.21 ± 0.77 and 12.99 ± 2.42 μg/ml, respectively. The water extract of *S. indica* exhibited higher anti-*H. pylori* activity than that of the ethanolic extract, and exhibited strong antioxidant activity on DPPH and $ABTS^{\cdot+}$ assay with the IC_{50} values of 8.169 ± 0.65 and 20.03 ± 1.33 μg/ml, respectively. Our results give new science-based evidence that *S. indica* fruit extract has a potential for treatment of gastritis. Pharmacological activities and toxicity in animal models should be further investigated.

KEYWORDS: *Shirakiopsis indica*, anti-inflammatory, cytotoxic, antioxidant, anti-*H. pylori*

INTRODUCTION

Gastritis is the inflammation of gastric mucosa. The pathological base of gastritis is dystrophy, inflammation, and unregeneration of gastric mucosa causing atrophy as the outcome of these symptoms [1]. The stomach has mechanisms to protect against hydrochloric acid and toxic agents. For example, gastric mucosa can control the acid and pepsin enzyme production, and the thickness of the gastric mucous coating can prevent acid toxicity in the stomach [2]. The inflammation of gastric mucosa or gastritis is a disorder of the stomach. There are two main causes of gastritis: *Helicobacter pylori* (*H. pylori*) infection and non-steroidal anti-inflammatory drugs (NSAIDs). *H. pylori* is a grade I carcinogenic bacterium that causes gastric cancer in humans [3], the most common non-erosive gastritis classified by International Agency for Research on Cancer (IARC), World Health Organization (WHO). In response to the *H. pylori* infection, the human body mechanism reacts to the inflammation site and generates reactive oxygen species (ROS) and reactive nitrogen species (RNS), i.e., nitric oxide and superoxide [4]. Nowadays, the treatment of gastritis varies according to the cause, but the primary problem has not been solved. Various herbs have been used in Thai traditional medicine to treat gastritis and peptic ulcers [5, 6]. The most common herb used for treating gastrointestinal symptoms is the fruit of *Shirakiopsis indica* (Sa-Mor-Ta-Lay in Thai). However, bioactivities of this herb have never been reported. The objectives of this study were to investigate the bioactivities of *S. indica* that influence the treatment of gastric inflammation, including cytotoxic activity against Kato III gastric

cancer cell line using the Sulforhodamine B (SRB) assay, antioxidant activity by DPPH and $ABTS^{\cdot+}$ assay, proinflammatory cytokines, i.e., nitric oxide (NO) by Griess assay, tumor necrosis-alpha (TNF-alpha), interleukin-6 (IL-6) using an ELISA kit assay, and anti-*H. pylori* using disc diffusion and minimal inhibitory concentration (MIC) assay. In addition, the chemical constituents of *S. indica* fruits were analyzed by performing LC-ESI-QTOF-MS/MS.

MATERIALS AND METHODS

Sample collection

Dried fruits of *Shirakiopsis indica* (Willd.) Esser (1000 g) were obtained from a local herbal medicine shop "Charoensuk Osod" in Nakorn Pathom, Thailand. Species identification, Verification Number 05698, was verified by the Herbarium in the Department of Biology, Faculty of Science, Naresuan University, Thailand, and this study was approved by the Biosafety Committee of University of Thammasat, Thailand under Biosafety Level 2 (BSL II, Number 011/2019).

Sample extraction and preparation

Water extraction

Dried *S. indica* fruits (92.49 g) were boiled in 500 ml of distilled water for 15 min, and the aqueous extract was filtered through a Whatman filter paper No. 1. Then, the filtrate was freeze dried using lyophilizer (Lyophilization systems Inc, USA).

Ethanolic extraction

Dried *S. indica* fruits (208.09 g) were crushed with in a mortar and macerated with 1 l of 95% ethanol for 3 days. The extract was filtered through Whatman filter paper No. 1 and re-macerated twice. Then, the combined extract was dried using a rotary evaporator (Buchi, Switzerland).

Cell culture

Human and murine macrophage cell lines

The Kato III gastric cancer cell line was obtained from the Riken Cell Bank (Tsukuba, Japan; catalog no. RCB2088). It was cultured in RPMI 1640 medium supplemented with 10% heated fetal bovine serum, 50 IU/ml of penicillin, and 50 μg/ml of streptomycin. For the anti-inflammatory activity, the murine macrophage RAW 264.7 cells (ATCC® TIB-71TM VA, USA) were cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% heated fetal bovine serum, 50 IU/ml of penicillin, and 50 μg/ml of streptomycin. The cells cultured were maintained at 37 °C in 5% CO_2 incubator (Shel lab, USA) at 95% humidity.

Bacterial strains and culture conditions

H. pylori (ATCC® 43504, USA) was obtained from the American Type Culture Collection. It was cultured on Columbia agar supplemented with 5% sheep's blood (RPD, Thailand) and incubated at 37 °C under microaerobic conditions (N_2, 85%; O_2, 5%; CO_2, 10%) using a gas generating kit. The bacteria were sub-cultured every three days in an anaerobic jar.

Antioxidant activities

DPPH radical scavenging assay

DPPH was measured by the method of Yamasaki et al [7] with a slight modification. DPPH solution (Fluka, USA) in absolute ethanol was freshly prepared and kept in a dark bottle protected from light. A 100 μl each of the water and ethanolic extracts and butylated hydroxytoluene (BHT) solutions of various concentrations (1, 10, 50, and 100 μg/ml) were placed in each well of a 96 well microplate. After that, a 100 μl of the DPPH solution was added to each well, and the plate was incubated in the dark at room temperature for 30 min. The radical scavenging activity was expressed as the number of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC_{50}). The antioxidant activity was calculated by its ability to inhibit the DPPH radical formation according to Eq. (1):

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100 \quad (1)$$

where $\text{Abs}_{\text{Control}}$ = absorbance of absolute ethanol; $\text{Abs}_{\text{Sample}}$ = absorbance of fruit extract.

$ABTS^{\cdot+}$ radical scavenging assay

The herbal extracts of 1, 10, 50, and 100 μg/ml concentrations were diluted. Then, $ABTS^{\cdot+}$ solution was prepared by diluting 7 mM $ABTS^{\cdot+}$ stock solution in distilled water with 2.45 mM potassium persulfate and then mixed at room temperature for 12–16 h. The solution was diluted with distilled water to obtain an absorbance at 734 nm in the range of 0.68–0.72. A 10 μl aliquots of diluted $ABTS^{\cdot+}$ solution and 90 μl of one of the *S. indica* extracts or standard Trolox were added into each well of the 96 well microplate, and mixed well. Then, the plate

was incubated for 6 min, and the absorbance (Abs) was measured at 734 nm by a microplate reader [8]. The antioxidant (radical scavenging) activity was calculated as percent inhibition of $ABTS^{\cdot+}$ radical formation according to Eq. (2):

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}})}{\text{Abs}_{\text{Control}}} \times 100 \quad (2)$$

where $\text{Abs}_{\text{Control}}$ = absorbance of absolute ethanol; $\text{Abs}_{\text{Sample}}$ = absorbance of fruit extract. The radical scavenging activity was expressed as the number of antioxidants (μg/ml) necessary to decrease the initial $ABTS^{\cdot+}$ absorbance by 50% (IC_{50}).

Anti-inflammatory activity on nitric oxide production and cytotoxicity

Anti-inflamatory activity on nitric oxide production

The anti-inflammatory activity of *S. indica* on nitric oxide production in murine macrophage RAW 264.7 cell line was determined by Griess assay [9–11]. The RAW 264.7 cells were diluted with the medium to obtain an optical density of 1×10^5. A 96 well microplate was seeded with 100 μl/well of the cell suspension and incubated at 37 °C in 5% CO_2 incubator (Shel lab, USA) at 95% humidity for 24 h. Next, 100 μl of fresh medium containing 10 ng/ml of lipopolysaccharide (LPS) (Sigma-Aldrich, USA) and various concentrations of test samples (1, 10, 30, 50, and 100 μg/ml) in DMSO (100 μl/well) were added to the wells and incubated for 24 h. The last step, after incubation for 24 h, nitric oxide production was determined with Griess reagent. Aliquots of 100 μl of the supernatant were transferred into a new 96 well microplate and mixed with 100 μl of Griess reagent. The absorbance (Abs) of the supernatants was measured with the microplate reader (Bio Tek, USA) at 540 nm. Inhibition was expressed as a percentage relative to the control samples, and dose-response curves were generated with Prism software (version 12) to determine the IC_{50} of the extracts. All the ethanol and water extract concentrations used in this anti-inflammatory activity on nitric oxide production were later tested for potential cytotoxicity in the MTT assay according to Eq. (3):

$$\% \text{ Inhibition} = \frac{(\text{Abs}_{\text{cont diff}} - \text{Abs}_{\text{sample diff}})}{\text{Abs}_{\text{cont diff}}} \times 100 \quad (3)$$

where $\text{Abs}_{\text{cont diff}} = \text{Abs}_{\text{control (+LPS)}} - \text{Abs}_{\text{control (−LPS)}}$; $\text{Abs}_{\text{sample diff}} = \text{Abs}_{\text{sample (+LPS)}} - \text{Abs}_{\text{sample (−LPS)}}$.

MTT assay for potential cytotoxicity

An MTT assay [10] was used to assess cytotoxicity of the *S. indica* extracts on RAW 264.7 cell line. The cells (1×10^5 cells/well) were incubated in 96 well microplates with various extract concentrations (1, 10, 30, 50, and 100 μg/ml) for 48 h. Then, the thiazolyl blue tetrazolium bromide (MTT) solution (Sigma-Aldrich, USA) was added to these wells and incubated for another 4 h. Next, the medium was removed, and isopropanol buffer (RCI Labscan, Thailand) with 0.04 M HCl was added to dissolve the formazan produced in the incubated cells. The absorbance of the formazan solution was measured with a microplate reader (Bio Tek, USA) at 570 nm. A dose-response curve was obtained by plotting the % inhibition values versus the extract concentrations. The extracts were considered non-toxic when cell survival was more than 70%. Cell survival was followed by determining % toxicity according to Eq. (4):

$$(\%) \text{ Toxicity} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}})}{\text{Abs}_{\text{control}}} \times 100 \quad (4)$$

where $\text{Abs}_{\text{control}}$ = absorbance of absolute ethanol; $\text{Abs}_{\text{sample}}$ = absorbance of fruit extract.

The inhibition of TNF-α and IL-6 production

The murine macrophage cell line (RAW 264.7 cell line) was incubated for 24 h with 5 ng/ml of LPS and *S. indica* extract. After 24 h, the proinflammatory cytokines, i.e., TNF-α and IL-6 productions, in each of the supernatants were determined by ELISA kit (ImmunoTools Co, Bangkok, Thailand) following the manufacturer's instructions. The contents of the 96 well microplate were determined with a microplate reader (Bio Tek, USA) at 420 nm [9, 11].

Anti-*Helicobacter pylori*

Disc diffusion method

H. pylori was incubated at 37 °C under microaerobic conditions for 3 days, and then adjusted to 0.5 McFarland standard equivalents. Filter paper discs (6 mm in diameter) were impregnated with the extract at the concentration of 1 mg/disc. The air-dried discs were placed onto the Columbia 5% sheep blood agar. Clarithromycin 1 mg/ml was used as a positive control. The plates were incubated as previously described for 3 days. The zone of inhibition was calculated by measuring the diameter of the inhibition zone 3 times, and the mean value was taken [6, 12].

Agar dilution method

The MIC values [13, 14] of the *S. indica* extracts were determined using the agar dilution method and following the Clinical and Laboratory Standards Institute guidelines. Each extract was serially diluted 2-fold in Columbia agar containing 5% sheep's blood, and then transferred into Petri dishes (Biomed, Thailand). The final concentrations of the extracts in the culture medium ranged from 31.25 to 2000 μg/ml. A colony of *H. pylori* ATCC 43504 was incubated in advance for 72 h. Thereafter, the *H. pylori* colonies were harvested and suspended in brain heart infusion. These bacterial suspensions were adjusted to 2.0 McFarland standard equivalents, transferred to multiple spots on each plate (3 μl per spot), and incubated at 37 °C for 72 h under microaerobic conditions. The MIC was defined as the lowest concentration of extract visibly able to inhibit the growth of *H. pylori* after an overnight incubation. In this experiment, the DMSO (RCI Labscan, Thailand) was used to dissolve the extract. All tests were conducted in triplicate.

Sulforhodamine B (SRB) assay

The gastric Kato III cancer cell line was seeded at a density of 5×10^4 cells in 96 well microplates and incubated overnight before being treated for 72 h with various concentrations (1, 10, 50, and 100 μg/ml) of the water and ethanolic extracts. Wells were then drained of liquid, and the cells were dried in an incubator for 72 h. The well plates were incubated at 37 °C in 5% CO_2 at 95% humidity. The cytotoxicity was analyzed after 7 days of treatment. The incubation was terminated by the addition of 100 ml per well of cold 40% (w/v) trichloroacetic acid (TCA) (Merck, Germany) left in the individual wells for 1 h at 4 °C. The plates were rinsed 5 times with water and dried. After that, 50 ml of 0.4% w/v SRB solution (Sigma-Aldrich, USA) in 1% acetic acid (Merck, Germany) was added to each well, and the plates were further incubated for 30 min at room temperature. SRB binds to the protein components of living cells previously exposed to TCA. After the SRB staining, the remaining (unabsorbed) dye was removed by washing 5 times with 1% acetic acid. The plate was dried, and the dried SRB residue was dissolved in 100 μl per well of 10 mM Tris base (Sigma-Aldrich, USA). The absorbance was read at 492 nm. A fitted dose-response curve was derived using linear regression to calculate the concentration of extract that killed 50% of the cells (IC_{50}) [15, 16]. The percentage of inhibition was calculated as follows (Eq. (5)):

$$\% \text{ Inb} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100 \qquad (5)$$

where $\text{Abs}_{\text{control}}$ = absorbance of 2% of DMSO; $\text{Abs}_{\text{sample}}$ = absorbance of fruit extract.

LC-ESI-QTOF-MS/MS analysis

An analysis of *S. indicum* extract was performed on an Agilent 1260 Infinity Series HPLC system (Agilent Technologies, Waldbronn, Germany) coupled with an Agilent 6540 Q-TOF-MS spectrometer (Agilent Technologies, Singapore) equipped with an electrospray ionization (ESI) interface. Chromatographic separation was on a Luna C18 (2) (150 mm × 4.6 mm i.d., 5 μm column (Phenomenax, Torrance, CA, USA). A 20 μl of sample solution (20 mg/ml) was injected into the LC system. The mobile phase consisted of (A) 0.1% v/v formic acid in water and (B) 0.1% v/v formic acid in acetonitrile. The elution was a linear gradient ranging from 5% A to 95% B lasting 30 min and holding at 95% B for 10 min before re-equilibration back to the initial condition lasting 5 min. The flow rate was set at 0.5 ml/min, and the temperature was controlled at 40 °C. The operating parameters for the ESI-MS detection were as follows: drying gas (gas N_2) flow rate (10.0 l/min), drying gas temperature (350 °C), nebulizer pressure (30 psig), capillary (3500 V), skimmer (65 V), octapole RFV (750 V), and fragmentor voltage (100 V) in positive mode. The mass range was set at m/z 100–1000 Da with a 250 ms/spectrum. For fragmentation, the non-targeted MS/MS mode was set with collision energies at 10, 20, and 40 V. All the acquisition and the analysis of data were controlled by Agilent LC-MS-QTOF MassHunter DataAcquisition Software version B.05.01 and Agilent MassHunter Qualitative Analysis Software B 06.0 (Agilent Technologies, USA), respectively. An analysis of each sample was performed in both positive and negative ionization mode [17].

Statistical analysis

The results are presented as mean ± standard error of means (SEM) of three independent experiments. For each experiment, samples at each concentration were tested in triplicate. The % inhibition was calculated using Microsoft Excel program. The IC_{50} values and statistical significance were calculated using one-way ANOVA, followed by Dunnett's multiple comparison test using the GraphPad Prism software (San Diego, CA, USA).

Table 1 Antioxidant activities (IC_{50}, μg/ml)of fruit extracts of *S. indica* ($n = 3$).

Fruit extract/control	$ABTS^{\cdot+}$ assay	DPPH assay
Water extract	$20.03 \pm 1.33^{*}$	$8.17 \pm 0.66^{*}$
Ethanolic extract	$66.47 \pm 1.28^{*}$	$50.95 \pm 1.47^{*}$
Butylated hydroxyuene (BHT)	5.66 ± 0.26	13.36 ± 0.18

Data were analyzed by one-way ANOVA and Dunnett's multiple comparison tests. Significant difference (*) is when $p < 0.05$ compared with the positive control (BHT).

Table 2 Anti-*H. pylori* and cytotoxic activities of *S. indica* fruit extracts ($n = 3$).

Fruit extract/ standard drug	Cytotoxic activity against Kato III	Anti-*H. pylori* ATCC 43504	
	(IC_{50}, μg/ml)	DDA[†] (mm)	MIC (μg/ml)
Water extract	21.76 ± 1.58	17.0 ± 0.0	2000
Ethanolic extract	3.21 ± 0.77	10.0 ± 0.0	1000
Curcuminoid	7.29 ± 1.90	NT	NT
Clarithromycin	NT	60.0 ± 0.0	0.5

[†] DDa = disc diffusion assay; NT = not tested.

RESULTS AND DISCUSSION

The extraction yields of the dried *S. indica* fruits extracted by water and ethanol were 3.77% and 1.28%, respectively. The water extract exhibited higher antioxidant activity than the ethanolic extract with the IC_{50} values in μg/ml of 20.03 ± 1.33 for ABTS assay and 8.169 ± 0.66 for DPPH assay (Table 1), while the ethanolic extract gave higher IC_{50} values by both assays. When compared with the positive control (BHT), the water extract exhibited 1.6-fold higher antioxidant activity by DPPH assay.

LPS is a composition of bacterial cell wall and functions as a stimulator for releasing pro-inflammatory cytokines. Therefore, nitric oxide indicates the capability in reducing inflammation of the extract. The determination of cell nitric oxide and toxicity were operated concurrently to ensure that the extracts did not reduce nitric oxide because the cells were dead and unable to produce nitric oxide [10]. The inhibition of nitric oxide production using Griess reaction assay was employed to determine the inhibitory activity, and the results showed that the ethanolic extract successfully reduced inflammation by inhibiting nitric oxideproduction at an IC_{50} of 12.99 ± 2.42 μg/ml (Table 3). The ethanolic extract of *S. indica* exhibited inhibitory activity on nitric oxide production 7.7-fold higher than the standard drug (acetaminophen). It was previously reported that high levels of nitric oxide in patients having the *H. pylori*-infected gastric mucosa might contribute to the development of gastric cancer [18]. Besides, the ethanolic extract of *S. indica* did not show significant levels of inhibitory activity on nitric oxide production compared with the prednisolone ($p > 0.05$). There was the correlation between high levels of nitric oxide and interleukin-6 production in *H. pylori*-infection gastritis [19]. On the other hand, the water and ethanolic extracts did not show the correlation between the inhibitory activity on nitric oxide, TNF-α, and IL-6 productions. The previous investigation by Bhanuz et al [6] revealed that the ethanolic extract of the *Amomum Krevanh* fruit showed higher anti-inflammatory activities than the water extract in both nitric oxide and interleukin-6 pathways. Additionally, the anti-*H. pylori* and antioxidant activities of the ethanolic extract were also higher than the water extract. The results of our study, from the inhibitory activity on nitric oxide production and cytotoxic activity against gastric cancer cell line, supported our goals of treatment of *H. pylori* infection or gastritis and reducing the risk of gastric cancer [20]. Both the water and the ethanolic extracts exhibited potent activity against *H. pylori* with an inhibition zone perimeter of 17.00 and 10.00 mm, respectively (Table 2). From the minimal inhibitory concentration (MIC) test, the ethanolic extract showed higher anti-*H. pylori* activity than the water extract, but the MIC values of both extracts against *H. pylori* were less than the standard drug (clarithromycin).

In the cytotoxicity assay, the ethanolic extract exhibited potent cytotoxic activity against the gastric cancer cell line with 2.27-fold higher than the curcuminoid drug (Table 2).

Five phorbol ester compounds found in the water extract are shown in Table 4. The characteristic loss of 151 Da from in-source fragmentation occurred at the interface of electrospray ionization (ESI) source for two of the five compounds. The two main compounds were found at m/z $[M+H]^+$ values of 540.26 (t_R 24.1 min) and 524.26 with the other three at t_R 26.9, 27.8, and 28.1 min, and the m/z 508.26 $[M+H]^+$ (t_R 32.5 min) resulted from the cleavage of methylaminobenzoate group. The structures of these compounds were related and they were isomeric. They could not be distinguished by mass spectrometry. The first of the compounds was found with m/z 522.25 $[M+H]^+$ and the second having two more protons, a structure of m/z 524.26 $[M+H]^+$, and a loss of 151 Da units. There might be a double bond somewhere in the phorbol part. Six known compounds losing 151 Da were found in the ethanol extract (Table 5). There

Table 3 Anti-inflammatory activities on NO, TNF-α, and IL-6 of *S. indica* fruit extracts ($n = 3$).

Fruit extract/standard drug	Anti-inflammatory (IC_{50}, μg/ml)			MTT assay
	Nitric oxide	TNF-α	IL-6	% Survival
Water extract	>100[a]	>100[a]	>100[b]	82.20
Ethanolic extract	12.99 ± 2.42[b]	>100[a]	>100[b]	77.85
Prednisolone	11.09 ± 0.27	0.11 ± 0.001	NT	82.84
Acetaminophen	> 100	>100	32.14 ± 2.69	61.29

The extract was considered non-toxic when cell survival >70%. NT = not tested. Data were analysed by one-way ANOVA and Dunnett's multiple comparison tests. Significant difference (a) is when $p < 0.05$ compared with the standard drug (Prednisolone), and (b) is when $p < 0.05$ compared with the standard drug (Acetaminophen).

Table 4 Structure elucidation of compounds found in the water extract of *S. indica* fruits using LC-ESI-QTOF-MS/MS operated in positive mode.

t_R (min)	m/z $[M+H]^+$	MS/MS*	Tentative identification	Formula	Error (ppm)
24.126	540.257	389.1951	Sapintoxin B	$C_{30}H_{37}NO_8$	4.06
24.543	219.174		(5beta,7beta,10beta)-3,11-Eudesmadien-2-one	$C_{15}H_{22}O$	1.56
26.922	524.2628	373.2, 313.1789	Sapintoxin A	$C_{30}H_{37}NO_7$	2.82
27.859	524.2624	373.1997	Sapintoxin C	$C_{30}H_{37}NO_7$	3.58
28.191	524.2629	373.200, 355.1891	a-Saipinine	$C_{30}H_{37}NO_7$	2.63
31.941	357.2054	300.1916	(17Z)-3,11-Dioxopregna-4,17(20)-dien-21-oic acid methyl ester	$C_{22}H_{28}O_4$	1.78
32.504	508.2678	357.20054, 339.1940, 315.1946	12-(2-N-methylamino benzoyl)-4a,5,20-trideoxyphorbol-13-acetate	$C_{30}H_{37}NO_6$	3.08

* means in source fragmentation.

were two compounds with m/z 540.26 $[M+H]^+$ at t_R = 24.1 and 26.3 min. The compound at t_R 26.3 min was not found in the water extract. We also detected a compound at m/z 522.25, found only at t_R 29.6 min. Three more compounds were also observed with m/z 389.19 $[M+H]^+$ (t_R 26.6 min), m/z 355.19 $[M+H]^+$ (t_R 29.3 min), and m/z 373.20 $[M+H]^+$ (t_R 30.52). These compounds were likely phorbol skeletons that lost their methylaminobenzoate groups. The negative ionization mode did not reveal any additional information about these six compounds. Other compounds besides these six were previously reported by Chumkaew et al [18].

CONCLUSION

Thai medicinal herb for treatment of gastritis plays an important role in Thai traditional healthcare services since it can be an effective substitution for Western medicine. Our study gave the first scientific evidence report on bioactivities of the fruit extracts of *S. indica* or Sa-Mor-Ta-Lay. The ethanolic extract of *S. indica* exhibited potent cytotoxic

Table 5 Structure elucidation of compounds found in the ethanolic extract of *S. indica* fruits using LC-ESI-QTOF-MS/MS operated in positive mode.

t_R (min)	m/z $[M+H]^+$	MS/MS*	Tentative identification	Formula	Error (ppm)
24.121	540.261	389.1975	Sapintoxin B	$C_{30}H_{37}NO_8$	−3.34
26.326	540.2607	389.1972	12-(2-N-methylamino benzoyl)-4a-deoxy-5,20-dihydroxyphorbol-13-acetate	$C_{30}H_{37}NO_8$	−2.79
26.917	524.2667	373.202	Sapintoxin A	$C_{30}H_{37}NO_7$	−4.62
27.774	524.2662	373.2021	Sapintoxin C	$C_{30}H_{37}NO_7$	−3.66
28.229	524.2663	373.2029	a-Saipinine	$C_{30}H_{37}NO_7$	−3.85
32.531	508.2718	357.2073	12-(2-N-methylamino benzoyl)-4a,5,20-trideoxyphorbol-13-acetate	$C_{30}H_{37}NO_6$	−4.79

* means in source fragmentation.

activity against gastric cancer cell lines and anti-inflammatory activity on nitric oxide production. Additionally, both the water and the ethanolic extracts showed antioxidant and anti-*H. pylori* activities. Therefore, *S. indica* has potent activities related to the symptoms of gastritis. However, further investigations into its mechanisms of action on pharmacological activities are still needed for future healthcare benefits.

Acknowledgements: This work was supported by Naresuan University research fund. The authors are grateful to the Faculty of Public Health, Naresuan University and the Faculty of Medicine, Thammasat University for providing logistic support and encouragement to carry out this research. Also, we thank Mr. Paul Freund and Prof. Dr. Duangrudi Suksang for reviewing the manuscript.

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