ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Research Article / JAPR / Sec. A
El-Sayed and Tawfik, 2024, 8 (1), 21-28

Journal of Advanced Pharmacy Research

Section A: Natural Products & Metabolomics

Phytochemical Profile and Analgesic Activity for Two *Syzygium* Species

Elsayed K. El-Sayed[1]*, Nashwa F Tawfik[2]

[1]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo, 11795, Egypt*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Helwan University. Ein Helwan, Cairo, 11795, Egypt*

***Corresponding author:** Elsayed K. El-Sayed, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Helwan University, Ein Helwan, Cairo, 11795, Egypt. Tel.: +20 01009146441*

E-mail address: elsayed_elsayed@pharm.helwan.edu.eg

Submitted on: 13-12-2023; Revised on: 01-01-2024; Accepted on: 02-01-2024

To cite this article: El-Sayed, S. K.; Tawfik, N. F. Phytochemical Profile and Analgesic Activity for Two *Syzygium* Species. *J. Adv. Pharm. Res.* **2024**, *8* (1), 21-28. DOI: 10.21608/aprh.2024.255361.1243.

ABSTRACT

Background: Genus *Syzygium* of the myrtle family comprises several aromatic species that are used for their aroma and edible fruits. Moreover, several *Syzygium* species exhibit antidiabetic, antifungal, anti-inflammatory, antibacterial, antioxidant, cytotoxic, antidiarrheal, anthelmintic, antinociceptive, analgesic and antivirus activities. **Objectives and methods:** The current study aimed to carry out the phytochemical screening for *S. malaccense* and *S. samarangense* leaves as well as isolation of the major phenolic compounds from 80 % aqueous methanol extract (AME) of *S. samarangense* using column chromatography and the compounds structures' were established by using chemical and physical methods. In addition, an investigation of the analgesic activity of the AME of two species was carried out for the first time using models of acetic acid-induced writhing test, hot plate test, and formalin-induced paw licking test in mice. **Results:** six phenolic compounds were purified from AME of *S. samarangense viz* methyl gallate **(1)**, gallic acid **(2),** quercetin-3-*O*-*β*-D-glucopyranoside or quercetin-3-*O*-*β*-D-galactopyranoside (**3),** quercetrin (**4),** ellagic acid **(5)** and quercetin (**6)**. **Conclusion:** two flavonoids, three phenolic acids, and one aglycone were isolated from *S. samarangense* leaves AME. Both extracts are safe for the experimental animals, and they showed significant antinociceptive and analgesic activities peripherally and centrally in a dose-dependent effect. Therefore, the two species can be used for their analgesic activity.

***Keywords**: Phytochemical screening, Phenolic compounds, Syzygium, Analgesic, Mice*

INTRODUCTION

The Myrtle family (*myrtaceae*) is one of the largest plant families, it comprises around 140 genera and 4600 –5800 species, most of which grow in tropical regions. [1,2] Genus *Syzygium* is one of Myrtaceae family comprising 1200–1800 species spread all over the world and some of its species are characterized by their edible fruits and aromatic constituents as well as, they have economic importance and can used as species[3]. *Syzygium malaccense* (L.) Merr. & L. M. Perry, commonly known as the Malay apple, is grown extensively around the world and it is an evergreen flowering tree with edible fruits, it is native to tropical Asia and Australia[4]. It is used traditionally as an antibacterial, astringent, and mildly hypoglycemic as well as, for the treatment of stomach aches, oral infections, and Children's mouth sores[5]. It was reported

that *S. malaccense* contains flavonoids from leaves[6], anthocyanins from fruits[7], as well as, organic acid derivatives were isolated from the leaves [8]. Moreover, the antioxidant, antimicrobial, anti-inflammatory, and antinociceptive activity of the leaf extract [6,7,8] were investigated. *Syzygium samarangense* (Blume) Merr. & L. M. Perry is an evergreen tree commonly known as wax apple or java apple, it is native to Andaman and Solomon Islands and cultivated in Asia and the Pacific zone, especially in Indonesia[9]. It has several traditional uses since its leaves can be used for cold, itches, and waist pain treatment, astringent, treating fever, and diarrhea, while the decoction of roots and barks was usually used in dysentery, women's disorders such as amenorrhea, menstrual flow stimulator, and inducing abortion [2]. It was reported that flavonoids, tannins, triterpenes, sterols phloroglucinols [2,10] were identified from the *S. samarangense* leaves. Moreover, flavonoids, pro-anthocyanins, and anthocyanins [2] were identified from the fruits. In addition, the antimicrobial activity of the fruit juice extract[11] and the antidiabetic, antioxidant, anti-inflammatory, anti-ulcerogenic, and hepatoprotective activities of the leaf extract were evaluated [12].

In the current study, we carry out the phytochemical screening for 80% aqueous methanol extract (AME) *S. samarangense* and *S. malaccense* as well as, isolation of the phenolic compounds for *S. samarangense* leaves in addition to investigating the analgesic activity for AME extract for both.

MATERIAL AND METHODS

Plant material

Syzygium malaccense (L.) Merr. & L. M. Perry and *Syzygium samarangense* leaves *(*Blume) Merr. & L. M. Perry were collected, from Mazhar Botanical Garden (June 2021). They were authenticated by Dr. Trease Labib, Senior Botanist at Mazhar Botanical Garden, Cairo, Egypt. Samples numbers (01Sma, 2021, and 01Ssa, 2021) were placed at the Department of Pharmacognosy herbarium, Faculty of Pharmacy, Helwan University, Cairo, Egypt.

General methods

^{1}HNMR spectra were measured at 400 on Bruker APX-NMR spectrometer. The data were stated as δ ppm values relative to the TMS. The ESI-MS data were measured on XEVO TQD triple quadruple LC/MS/ MS. (Waters Corporation, Milford, MA01757 United States). Polyamide (Fluka Chemie, Switzerland), cellulose (E. Merck-Darmstadt, Germany), and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for CC (column chromatography) as stationary phase while ethanol, aqueous ethanol, and aqueous methanol were used as mobile phase. Whatman No. 1 mm papers (Whatman Ltd., Maidstone, Kent, England) were used in PC (paper chromatography). *n*-Butanol: acetic acid: water, 4-1-5 upper layer (BAW, S_1) and acetic acid: water 15-85 (HAc, S_2) were used for elution in the first and second run respectively. For the detection of the phenolic compounds on the PC chromatograms, UV light (UV lamp, VL-215 LC, Marne La Vallee, France), Naturstoff reagent, and $FeCl_3$ were used. The solvents used are of analytical grade and supplied by El Nasr Pharmaceutical Chemicals Company, Egypt; acetic acid (CID Pharmaceutical Co. Egypt), indomethacin (El-Nile Co. Egypt), and tramadol (Amadol® ampoule) (Adwia Pharmaceuticals Co., Egypt).

Experimental animal

Male Swiss albino mice (20-25 g) were used in the current study. Mice were purchased from the breeding unit of the Egyptian Organization of Biological Products and Vaccines located in Helwan, Egypt. Mice were housed under standard conditions (12 h light/dark cycle and temperature at 23 ±1 ◦C), and were given food and water *ad libitum*. The experimental protocol for animal handling and all related procedures were approved by the animal care and use committee at the Faculty of Pharmacy, Helwan University (Protocol no. 16 A2023).

Phytochemical screening for the leaves AME of *S. samarangense* and *S. malaccense*

Powdered air-dried leaves of the two species (200 g for each) were extracted with 80% aqueous methanol and then filtered and dried under reduced pressure. The remaining residue was subjected to qualitative phytochemical screening to detect the presence of different classes of the phytoconstituents as flavonoids, tannins, coumarins, alkaloids, cardiac glycosides, saponins, carbohydrates and/ or glycosides, sterols and/or triterpenes and anthraquinones[13, 14]. Moreover, the presence of essential oil was done on the fresh plant sample.

Purification of the phenolic metabolites from *S. samarangense*

The powdered leaves of *S. samarangense* (1.0 kg) were extracted by reflux using 80% aqueous methanol (4.0 L x 3, 60°C), yielding 160 g of crude extract. Defatting of the dried extract was done under reflux for 5.0 h using *n*-hexane (2L x 4, 60°C) to yield 50 and 90 g of *n*-hexane and defatted aqueous methanol residue, respectively. 20.0 g of the defatted AME was used for biological activity while the remaining extract (40.0 g) was dissolved in 10.0 % AM and subjected to fractionation using polyamide CC and eluting using step gradient elution (10%MeOH/H_2O -100%MeOH to give eleven fractions. The collected fractions were subjected to PC using S_1 and S_2 as well as spraying with Naturstoff

reagent and $FeCl_3$ to collect similar fractions (FrI-V). FrI (10 %MeOH/H_2O) was mainly sugars and salts and contains small amounts of phenolic compounds. FrII (20-30% MeOH/H_2O was purified by cellulose CC eluted with 10 – 50 % aqueous methanol to give compounds **1** (15 mg) and **2** (10 mg). FrIII (40-50 % aqueous methanol) was subjected to cellulose CC eluted with aqueous methanol (0-100%) to give two subfractions (i and ii). Each sub-fraction was further purified using Sephadex LH-20 (1:1 aqueous methanol) yielding pure samples of compounds **3** (20 mg) and **4** (15 mg).

Fr V was subjected to a cellulose column using aqueous methanol (20 - 90%) to afford the crude sample of **5** and **6** which was further purified using cellulose column (50 % ethanol) to give pure samples of **5** (10 mg) and **6** (8 mg). Purity of the compounds was established using PC (S_1 and S_2) as well as visualizations under UV light and spraying with Naturstoff reagent and $FeCl_3$ reagent.

Complete acid hydrolysis

1-2 mg of the sample was heated with 1.5 N HCl in 50% aqueous MeOH for 2 h at 100 ºC under reflux then the reaction mixture was examined using TLC to detect aglycone (in organic phase) and sugars (in aqueous phase) against available authentic references.[15.]

Compounds 1 and 2

They were purified as off-white amorphous powders. They gave a pale blue color with $FeCl_3$. Their -ve ESI-MS showed a molecular ion peak at *m/z* $[M-H]^-$ 169.0023 and 183.2470 for compounds **1** and **2** respectively.

Compound 3

It is a yellow amorphous powder with R_f value of S_1(0.53); S_2 (0.48). It showed a dark purple spot under UV light (λ_{max} 365) changed to an orange color upon spraying with Naturstoff reagent. -ve ESI-MS: *m/z* 463.0920 $[M-H]^-$, 300.9434 [aglycone- H]$^-$. ^{1}HNMR (400 MHz, DMSO-d_6) δ_H:12.40 (1H, s, H-bonded OH-5), 7.87 (br s, H-2`), 7.44 (dd, *J* = 6.8 Hz, br s, H-6`), 6.83 (d, *J* = 6.8 Hz, H-5`), 6.40 (br s, H-8), 6.20 (brs,H - 6), 5.34 (brd, H-1``), 3.38 - 3.27 (remaining sugar protons).

Compound 4

It was purified as yellow amorphous powder with R_f value of S_1(0.73); S_2 (0.51) as well as, it displays dark purple fluorescence under long UV light (λ_{max} 365) which changed to orange color after praying with Naturstoff reagent. -ve ESI-MS: *m/z* 447.1517 $[M-H]^-$. ^{1}HNMR (400 MHz, DMSO-d_6) δ_H:12.64 (1H, s, H-bonded OH-5), 7.67 (br s, H-2`), 7.54 (dd, *J* = 6.8 Hz, br s, H-6`),, 6.85 (d, *J* = 7.6 Hz, H-5`), 6.39 (br s, H-8), 6.21 (brs,H - 6), 5.25 (brd, H-1``), 3.98 (br d, H-2``), 3.77-3.15 (m, H-3``, H-4``, H-5``), 0.80 (br d, H-6``).

Compound 5

It was purified as yellow amorphous powder with R_f value is 0.73 and 0.02 in S_1 and S_2, respectively. It displays yellow colour fluorescence under long UV light, which turned to orange colour by spraying with Naturstoff reagent. -ve ESI-MS: *m/z* 301.0662[M-H]).

Compound 6

It was isolated as an off-white amorphous powder with R_f value of S_1(0.24); S_2 (0.02). It gave buff fluorescence with UV light and changed to blue colour upon spraying with $FeCl_3$.-ve ESI-MS: *m/z* 300.9978[M-H]).

In vivo experiments

Determination of median lethal dose (LD_{50})

The AME of *S. malaccense* and *S. samarangense* were administered orally, using different doses up to 2000 mg/kg. 48 h after treatment, any signs of toxicity were recorded, and the number of deaths was counted[16].

Analgesic activity

Acetic acid-induced abdominal writhing in mice

The AME's analgesic activity was assessed using the methodology outlined by Ping et al.[17] using acetic acid to induce abdominal writhing behavior in mice. Mice that had fasted for the night were divided into eight groups (n = 6). Group 1: The control group received the vehicle (0.9% NaCl). Group 2: Standard group received indomethacin (10 mg/kg, i.p.). Groups 3-5: treated with *S. malaccense* 250, 500 and 1000 mg/kg orally. Groups 6-8: treated with *S. samarangense* 250, 500, and 1000 mg/kg orally. One hour following treatment with indomethacin or extracts, 10 mL/kg of acetic acid (0.6 % v/v) was administered intraperitoneally for all mice in all groups. Over thirty minutes, the number of abdominal contractions (writhing behavior) was counted. According to the following formula: the percentage inhibition of abdominal contractions was used as an indicator of the analgesic activity.

% Inhibition = MWC–MWT/ MWC x 100

MWC = Mean number of writhing for the control group
MWT= Mean number of writhing for the tested group

Formalin-induced paw licking in mice

According to the method of Chang et al[18], a 20 microliter of formalin solution (5%) was injected via a subcutaneous route in the right hind paws of the mice for pain induction. Vehicle, standard (indomethacin, 10 mg/kg; i.p.), or oral AME extracts of *S. malaccense* and *S. samarangense* at doses of 250, 500, and 1000 mg/kg

were administered 1 h before the injection of formalin. After being injected with formalin, the total time (measured in seconds) that the injected paw was licked, and bit was recorded for five minutes (early phase) and twenty to thirty minutes (late phase).

Hot plate test in mice

In the current study, the central analgesic activity was assessed using the hot plate method according to the method of Mady et al.[19]. The apparatus contains a hot plate surface with a temperature of 55 ± 1°C and a Plexiglass cylinder. Each mouse was introduced to the cylinder with a paw touching the hot plate surface. The reaction time (Time in seconds (s) required for paw licking or humping) was recorded. Eight groups were allocated as described in the above section. The reaction time was recorded before and after 15, 30, 60, and 90 minutes (min) following administration of vehicle, standard, or different doses of extracts. Twenty seconds as a cut-off time was used as a finishing point of the analgesic action and to avoid paw tissue damage.

Statistical analysis

All results were expressed as mean ± SEM. Prism 8 software (GraphPad Software Inc., California, USA) was utilized to compare between groups using one-way analysis of variance (ANOVA) followed by Tukey post-hoc tests. The value $p < 0.05$ was considered as significant.

RESULTS

Phytochemical analysis

Based on the qualitative chemical tests on the AME of *S.malaccense* and *S. samarangense* leaves **(Table 1),** it was found that both species showed the presence of essential oils, flavonoids, tannins, coumarins, carbohydrates and/or glycosides, and sterols and/ or triterpenes while saponins, alkaloids, cardiac glycosides, and anthraquinones are absent in both species.

Moreover, 2D-PC for AME of both species displayed many spots with shiny violet, buff and dark purple under UV light which turned to orange, yellowish green, and blue upon spraying with Naturstoff reagent and $FeCl_3$ reagent. These findings confirm the presence of phenolic constituents including flavonoids, tannins, and phenolic acids in the AME of both species.

Compounds 1 and 2

Based on their chromatographic properties, comparison with authentic samples, and comparison of their –ve ESI/ MS with previously published data, compounds **1** and **2** are identified as gallic acid and methyl gallate respectively.

Compounds 3 and 4

They displayed chromatographic properties characteristic of quercetin 3-*O*-glycosides[20]. On complete acid hydrolysis, they gave quercetin in the organic phase using comparative paper chromatography (Co-PC) as well as, glucose and rhamnose were detected in the aqueous phase of 3 and 4 respectively (Co-PC). ^{1}HNMR spectral data for the two compounds showed ABX system for three aromatic protons at δ_H 7.87,7.44. 6.83 (Compound 3) and 7.67,7.54. 6.85 (Compound 4 for H-6', H-2' and H-5', respectively, in addition to AM system for H-8 at $\delta_H \approx 6.40$ and H-6 at $\delta_H \approx 6.20$, which confirms that the aglycone of both compounds is quercetin [20]. Moreover, in the case of 3 the presence of anomeric protons at δ_H 5.34 (H-1") confirms the presence of glucose or galactose while in the case of 4, the presence of anomeric protons at δ_H 5.25 together with broad doublet signal at δ_H 0.8 support the presence of rhamnose moiety. Final confirmation was established from their –ve ESI /MS, which displayed a molecular ion peak at *m/z* 463.0920 $[M-H]^-$ corresponding to quercetin aglycone with one glucose or galactose moiety for **3** and *m/z* 447.1517 $[M-H]^-$ for quercetin with one rhamnose moiety in case of **4**. Therefore, based on the chromatographic properties, acid hydrolysis, ^{1}HNMR, -ve ESI/MS as well as, comparison with previously published data, [21], compound **3** may be quercetin-3-*O*-*β*-D-glucopyranoside (isoquercetin) or quercetin-3-*O*-*β*-D-galactopyranoside while compound **4** identified as quercetin 3-*O*- *α*- L- $^1C_4$- rhamnopyranoside (Quercitrin).

Table 1. Phytochemical screening of *S.malaccense* and *S. samarangense* leaves

Constituents	Results	
	S.malaccense	***S. samarangense***
Essential oils	+	+
Carbohydrates and/or glycosides	+	+
Sterols and/ or triterpenes	+	+
Flavonoids	+	+
Tannins	+	+
Coumarins	+	+
Alkaloids	-	-
Cardiac glycosides	-	-
Saponins	-	-
Anthraquinones	-	-

(+), present; (-), not detected

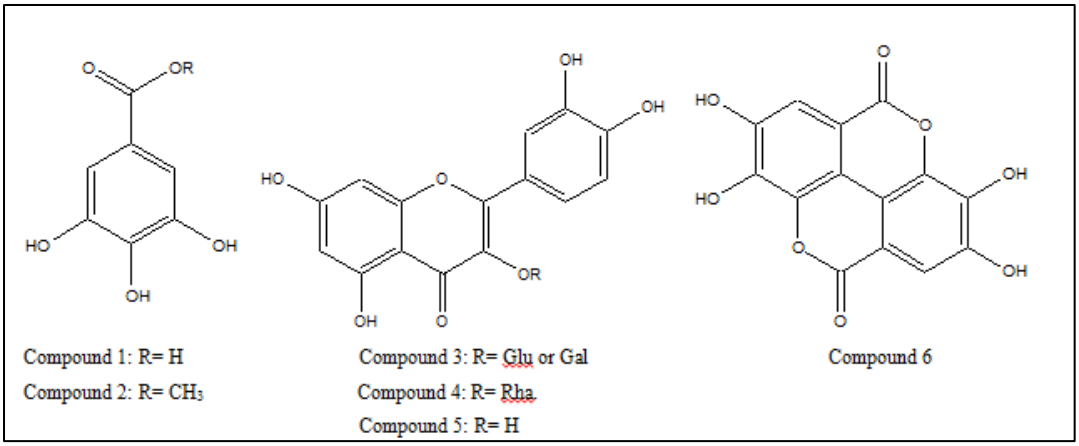

Figure 1. The compounds identified from *S. samarangense* AME. Glu: glucose; Gal: galactose; Rha: rhamanose.

Compound 5

Based on its chromatographic properties, -ve ESL/MS, and comparison with authentic sample and literature data, it was identified as quercetin [20].

Compound 6

Regarding its chromatographic properties (R_f-values and response with different spray reagents), addition to Co-PC versus authentic sample by using convenient solvent systems as well as, its -ve ESL/MS, it was identified as ellagic acid [22].

In vivo experiments

Acute toxicity (LD50)

The doses of the extracts up to 2 g/kg did not reveal any signs of toxicity or mortality, therefore the doses of 250,500 and 1000 mg/kg were the selected doses used in the study.

Acetic acid-induced abdominal writhing in mice

This test was used to evaluate the peripheral analgesic activity of *S. malaccense* and *S. samarangense*. Oral administration of the AME of *S. malaccense* (250,500 and 1000 mg/kg) and *S. samarangense* (250,500 and 1000 mg/kg) showed a significant ($p < 0.05$) analgesic activity in a dose-dependent manner evidenced by a significant decrease in writhing number by 28.5%, 48.8%, 62.4%, 36.6%, 52.9%, and 69.5% respectively as compared to the control group **(Figure 2A).** In the same context, the indomethacin standard group significantly ($p < 0.05$) decreased the number of writhing by 81% when compared to the control group. Notably, treated groups with 250 and 500 mg/kg for both extracts showed a significant change when compared to the indomethacin group. Interestingly, the dose of 1000 mg/kg was non-significant from the standard indomethacin group.

Formalin-induced paw licking in mice

As represented in **Figure (2B, C)**, in a dose-dependent manner, the AME of *S. malaccense* and *S. samarangense* significantly ($p < 0.05$) decreased the time of paw licking. At the early phase, the AME of *S. malaccense* (250,500 and 1000 mg/kg) and *S. samarangense* (250,500 and 1000 mg/kg) significantly ($p < 0.05$) decreased paw licking time by 22.6%, 34.2%, 44.1%, 24.4%, 38.1%, 47.4% respectively as compared to the control group. Moreover, indomethacin-treated mice significantly ($p < 0.05$) declined paw licking time by 52.7% as compared to the control mice **(Figure 2B)**. Regarding, the late phase, the time of paw licking was significantly ($p < 0.05$) decreased by approximately 44%, 11.3%, 23.5%, 28.6%, 19.7, 33.3%, 35.7% for indomethacin, *S. malaccense* (250,500 and 1000 mg/kg) and *S. samarangense* (250,500 and 1000 mg/kg) respectively as compared to the control group **(Figure 2C)**.

Hot plate test in mice

The central analgesic efficacy of two *Syzygium* species was evaluated using the hot plate test. After 90 minutes, treatment with standard caused a significant ($p < 0.05$) increase in the reaction time by 4.5 folds compared to the control group. Treatment with *S. malaccense* in doses 250, 500, and 1000 caused a significant ($p < 0.05$) increase in reaction time by 1.8 folds, 2.2 folds, and 2.6 folds, respectively, compared to the control group. Meanwhile, treatment with *S. samarangense* in doses 250, 500, and 1000 caused a significant ($p < 0.05$) increase in reaction time by 2 folds, 2.7 folds, and 3.1 folds, respectively, compared to the control group. The highest reaction time was observed ($p < 0.05$) in a dose of 1000 mg/kg **(Table 2).**

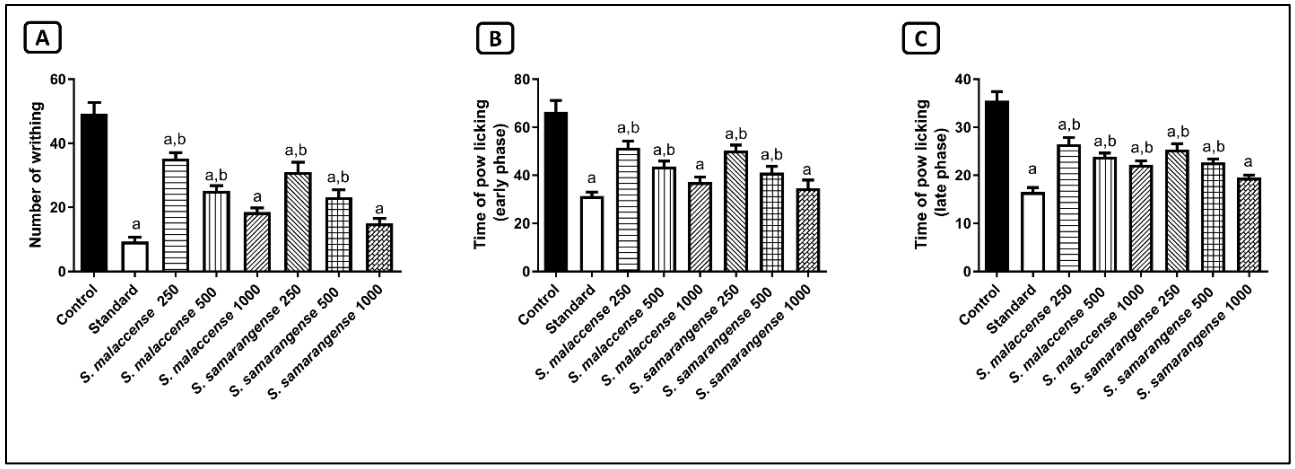

Figure 2. Effect of AME of *S. malaccense* and *S. samarangense* on: (A) Numbers of writhing induced by acetic acid. (B): Paw licking time (early phase). (C) Paw licking time (late phase). Results were expressed as: Mean ± SE, (n = 6); a: significant from control and b: significant from standard.

Table 2. Effect of AME of *S. malaccense* and *S. samarangense* on the reaction time in hot plate test.

Groups	Reaction time (s) before administration of AME	Reaction time (s) after 15 min of AME	Reaction time (s) after 30 min of AME	Reaction time (s) after 60 min of AME	Reaction time (s) after 90 min of AME
Control	4.00 ± 0.26	3. 67 ± 0.21	4.00 ± 0.26	3.83 ± 0.17	4.17 ± 0.31
Standard	3.83 ± 0.31	5.17 ± 0.31 [a]	8.83 ± 0.31 [a]	15.00 ± 1.21 [a]	18.67 ± 1.52 [a]
***S. malaccense* 250**	4.33 ± 0.33	3.83 ± 0.31 [b]	5.17 ± 0.30 [b]	7.17 ± 0.48 [b]	7.67 ± 0.33 [a,b]
***S. malaccense* 500**	4.00 ± 0.37	4. 50 ± 0.22	5. 67 ± 0.33 [b]	8.17 ± 0.54 [a,b]	9.17 ± 0.54 [a,b]
***S. malaccense* 1000**	4.00 ± 0.25	4.67 ± 0.33	6.00 ± 0.58 [a,b]	10.00 ± 0.58 [a,b]	10.67 ± 0.49 [a,b]
***S. samarangense* 250**	3.84 ± 0.32	4.17 ± 0.31	5.50 ± 0.43 [b]	8.00 ± 0.37 [a,b]	8.33 ± 0.33 [a,b]
***S. samarangense* 500**	4.00 ± 0.36	4.5 ± 0.22	6.00 ± 0.37 [a,b]	9.83 ± 0.48 [a,b]	11.17 ± 0.70 [a,b]
***S. samarangense* 1000**	3.83 ± 0.31	4.83 ± 0.31	7.88 ± 0.58 [a]	11.33 ± 0.80 [a,b]	13.00 ± 0.68 [a,b]

Results were expressed as Mean ± SE, (n = 6); a: significant from control and b: significant from standard

DISCUSSION

In the current research we evaluate the analgesic activity together with the phytochemical analysis of the *S. malaccense* and *S. samarangense* leaves AME. The presence of flavonoids, tannins, sterols and/or triterpenes, and carbohydrates and/or glycosides was detected by phytochemical screening in both species. In addition, numerous phenolic chemicals, including flavonoids and tannins, which are thought to be useful therapeutic agents for the treatment of numerous ailments, were found to be present, according to 2D-PC [23]. The analgesic efficacy of both AME species was assessed in vivo through the application of thermal and chemical stimuli, including formalin and acetic acid.

Acetic acid plays a major part in the mechanism of pain by stimulating the biosynthesis of prostaglandin and cyclooxygenase, which in turn releases arachidonic acid. This is applied to the mouse model of acetic acid-induced abdominal writhing.[24] Increased production of both prostaglandin E2 and F2 α at the peritoneal cavity stimulates the pain receptors and causes pain sensation that appears in the form of writhing[25]. Additionally, it is thought that acetic acid works indirectly by using endogenous mediators like prostaglandins, histamine, and serotonin to stimulate peripheral nociceptive neurons[26]. At all doses, treatment with AME of both species significantly decreased the pain sensation in comparison with the control group. More interestingly, treatment with high doses (1000 mg/kg) exhibited a non-

significant antinociceptive effect in comparison with the standard group. The ability of AME to prevent the release of inflammatory mediators or impede the activity of peripheral cyclooxygenases may account for its analgesic and antinociceptive effects. In the meanwhile, there are two phases of pain in the formalin-induced paw-licking model in mice, which is thought to be a persistent pain model. Neurogenic pain is the term used to describe the initial phase (0–5 min), while inflammatory pain is the term used to describe the second phase (20–30 min) [27]. Our data show that, in comparison to the control group, treatment with AME at the studied doses considerably reduced the licking time in the two stages. As previously noted, C fiber activation had a major role in the early phase of pain, but the late phase of pain was primarily caused by a combination of peripheral tissue inflammation and functional alterations in the spinal cord's dorsal horn [28]. Conversely, the hot plate test is thought to be a paradigm that is selective in assessing central analgesic action. This hypothesis is predicated on the heat stimulus's capacity to activate peripheral nerve endings and produce impulses that go via the spinal cord to the brain. This approach is frequently used to screen for medications that block centrally sourced pain, such as tramadol, which are centrally acting opiate analgesics [29, 30]. Consequently, the results obtained validate the AME's central antinociceptive impact and its method of action, which partially involves the opioid-mediated system. As a result, the current study's findings showed that AME exhibited strong analgesic activity, both centrally and peripherally. This effect may have been caused by the high phenolic content of AME, which may inhibit the cyclooxygenases, lipoxygenases, nitric oxide synthases, and arachidonic acid metabolic pathway[31].

CONCLUSION

The current work shows that *S. malaccense* and *S. samarangense* leaves AME reach in polyphenols. Six phenolic compounds were purified from AME of *S. samarangense viz* methyl gallate **(1)**, gallic acid **(2),** quercetin-3-*O*-*β*-D-glucopyranoside or quercetin-3-*O*-*β*-D-galactopyranoside (**3),** quercetrin (**4),** ellagic acid **(5)** and quercetin (**6**). Moreover, this study endorses the analgesic activity of *S. malaccense* and *S. samarangense* leaves AME. which may be correlated to the polyphenolic contents.

Acknowledgements

Our deepest thanks to Professor Fatma Moharram, Department of Pharmacognosy, Faculty of Pharmacy, Helwan University for revising the manuscript.

Funding Acknowledgment

No external funding was received.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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