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RESEARCH ARTICLE

Wound Healing Potential of Ya-RakSa-Phlae: a Traditional Thai Herbal Recipe

DENNAPA SAELOH[1,2], PHANSORN RATTANAMA[3,4], AUEMPHON MORDMUANG[5,2], JULALAK CHOKPAISARN[3,2]*

[1]*Faculty of Medical Technology, Prince of Songkla University, Songkhla 90112, Thailand*

[2]*Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*

[3]*Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand, Email: julalak.c@psu.ac.th*

[4]*Faculty of Public Health, Chalermkarnchana University, Chang Sai, Phra Phro, Nakhon Si Thammarat, 80000*

[5]*School of Medicine, Walailak University, Tha Sala, Nakhon Si Thammarat, 80160, Thailand*

** Corresponding Author*

ABSTRACT

Aim: This study was aimed to preliminarily investigate the related-wound biological and wound healing properties of the ethanolic extract of Ya-RakSa-Phlae recipe (E-YRSP) to prove its traditional property.

Methods: Phytochemical screening on total phenolics of E-YRSP was observed and GC-MS was also performed to evaluate the chemical profile of the extract. Moreover, biological properties related to wound healing activity such as anti-bacterial, anti-oxidant, anti-inflammatory, and wound healing activities were carried out.

Results: E-YRSP possessed anti-bacterial activity with MIC 16-1,024 µg/mL against both Gram-positive and Gram-negative bacteria. Moreover, it was found to inhibit inflammation with IC50 up to 1,000 µg/mL and oxidation with IC50 ranged from 6-20 µg/mL. It was noted that activities of E-YRSP on anti-infection, anti-inflammation, and anti-oxidation markedly resulted in a competent wound healing efficacy. Phenolic compounds were abundantly found in E-YRSP as a large group of phytochemical constituents including catechin, rutin, demethoxycurcumin, luteolin, curcumin, and mangostin which might be a bioactive agent for wound healing promotion.

Conclusion: This study has confirmed that YRSP obviously possessed strong wound healing activity as well as other biological properties associated with wound healing including, anti-oxidation, anti-inflammation, and anti-bacteria. Therefore, it is worthwhile to further study on its mechanisms on wound healing process and also to develop as an alternative treatment for wound treatment.

KEYWORDS:
Anti-bacteria, Anti-inflammation, Anti-oxidation, Wound healing, ya-raksa-phale

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INTRODUCTION

Wound healing is a normal human response which suddenly occurs after the injury. However, an inappropriate response of the healing process can result in the problem of chronic wounds (1-2). There are several risk factors that can delay the healing process, including local infections, excessive inflammatory mediators, as well as oxidative stress. Therefore, successive wound therapies always need the combination of treatments to overcome these causing factors (3-4). Nowadays, use of traditional herbal medicine for wound treatment is occurring popularly. It is getting to be important, showing a huge impact on the global health care system (5).

As traditional Thai medicine, there is an herbal recipe that has been utilized by Thai folklore doctors for treatment of skin diseases and wounds, especially chronic wounds named as “Ya-RakSa-Phlae” (YRSP). It consists of six medicinal plants including, Curcuma longa L., Areca catechu L., Garcinia mangostana L., Zingiber montanum (Koenig), Uncaria gambier Roxb., and Oryza sativa L. Several studies have been exhibited that each plant in the recipe has potent wound healing activity (6-8), anti-inflammatory (9-10), anti-oxidant (11-12) as well as anti-bacterial activities (13-15). YRSP has been traditionally used for wound treatment for a long time,

however, there are no scientific researches confirming its traditional use. Therefore, this study was aimed to preliminarily investigate the related-wound biological and wound healing properties of the ethanolic extract of Ya-RakSa-Phlae recipe (E-YRSP) in in vitro experiments to prove its traditional property.

MATERIALS AND METHODS

Plant materials and extraction of Ya-RakSa-Phlae (YRSP)

YRSP is an herbal recipe for treatment of skin diseases composed of six medicinal plant parts including, rhizome of Curcuma longa L., seed of Areca catechu L., peel of Garcinia mangostana L., rhizome of Zingiber montanum (Koenig), resins of Uncaria gambier Roxb., and seed of Oryza sativa L. All the materials are deposited as reference voucher specimens at the Faculty of Traditional Thai Medicine, Prince of Songkla University, Hat Yai, Songkhla, Thailand. For the extraction, all dried materials were grounded into fine powder and the equal amount (100 mg) of each plant powder was mixed together with 500 mg of the Oryza sativa powder. The powdered formulas (100 g) were submitted to solvent extractions with 95% ethanol in the ratio of 1:1 for seven days. After filtration through a Whatman No. 1 filter paper, the solution was evaporated to dryness using a rotary evaporator. Yields of Ya-RakSa-Phlae ethanolic extract (E-YRSP) was calculated according to the below formula. The sample was kept in a sterile bottle at 4° C until use.

% yield = (the weight of the extract / the weight of the crude herb powder) × 100

Determination of total phenolic content

Total phenolic content of the extract was estimated using the Folin-Ciocalteu method as described earlier. The extract was dissolved in DMSO and diluted to a starting concentration of 1,000 mg/ml. The solution (10 µL) was mixed with 150 µL of 20% Folin-Ciocalteu's reagent dissolved in sterile distilled deionized water. After 3 min of dark incubation, 6% (w/v) sodium bicarbonate was added and further incubated for 2 hour in the dark. The absorbance was measured at 765 nm using an UV-visible spectrophotometer. Gallic acid was used as the reference standard and the results were expressed as micrograms of gallic acid equivalent per gram of fresh weight (mg of GA/g of extract) (16).

Gas chromatography-mass spectroscopic analysis

Ethanolic extract of YRSP (E-YRSP) was analysed for the presence of phytochemical components by Gas chromatography-Mass spectroscopy (GC-MS) technique using a Liquid chromatography-quadrupole time-of-flight mass spectrometer (LC-QTOF MS) equipped with a Hypersil™ ODS column (4.6×250 mm, 5 µm). The temperature was programme from 40ºC to 325ºC. The inert gas helium (99.9%) was used as carrier gas with the flow rate of 13 l/min. The sample (2µl) was injected through the injector. Mass spectra were taken at 70eV and the total GC running time was 30 minutes. For GC-MS detection, the spectrum of E-YRSP was detected with the spectrum of the compound stored in the National Institute Standard and technology (NIST), through comparison of peaks, retention time, as well as compound matching.

In vitro DPPH free radical scavenging assay

The free radical scavenging activity of E-YRSP was performed using stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay as described before (16). YRSP was dissolved in DMSO by two-fold serial dilution method and then gently mixed with DPPH solution (180 µL). After 15 min of incubation in the dark, the reaction was determined at 517nm using a UV-Vis spectrophotometer. The reaction was carried out in triplicates with trolox as the standard control. The result was shown as the half maximal inhibitory concentration (IC50) by plotting inhibition percentage against extract concentration.

In vitro ABTS free radical scavenging assay

ABTS free radical scavenging assay was assessed according to the previous method (16). ABTS solution was oxidised with potassium persulfate for 16 h in the dark under the room temperature. After 16 h, the oxidised ABTS solution was further diluted with phosphate buffer saline pH 7.4 to achieve an absorbance of 0.70 ± 0.02 at 730 nm. For the measurement, E-YRSP was serially dissolved with DMSO by two-fold dilution method and then gently mixed with the ABTS+ solution for six mins in the dark. Then, the reaction was read at 734 nm using an UV-visible spectrophotometer. The reaction was carried out in triplicates with trolox as the standard control. The result was shown as the half maximal inhibitory concentration (IC50) by plotting inhibition percentage against extract concentration.

In vitro nitric oxide radical scavenging assay

No scavenging assay was determined as previously described. Briefly, sodium nitroprusside in phosphate buffer saline (20 µL) was gently mixed with different concentrations of E-YRSP (40 µL) and then incubated in the dark for 180 min at room temperature. After incubation, 30 µL of 1% sulphanilamide in 2.5% phosphoric acid was added and left in the dark for another 8 min. Then, the sample was mixed with 30 µL of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid and 60 µL of phosphate buffer saline. The mixture was incubated in the dark for 8 min and the absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with NED was read at 546 nm using a UV–VIS spectrophotometer. The reaction was carried out in triplicates with catechin as the standard control. The result was shown as the half maximal inhibitory concentration (IC50) by plotting inhibition percentage against extract concentration (17).

Anti-bacterial activity of E-YRSP

Bacterial strains and culture

The bacterial strains were obtained from Excellence Research

Laboratory on Natural Products, Faculty of Science and Natural Product Research Center of Excellence, Prince of Songkla University, Thailand. Staphylococcus aureus ATCC 25923, S. epidermidis ATCC 35984, methicillin-resistant S. aureus (MRSA), Bacillus subtilis, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, E. coli O157-H7, Acinetobacter baumannii ATCC 19606, Klebsiella pneumoniae ATCC 700603, and Pseudomonas aeruginosa ATCC 27853 were subjected for testing antibacterial activities of YRSP. The tested bacterial were cultured on tryptic soy agar (TSA, Becton Dickinson, Difco, France) at 37 °C for 18–24 h. The bacterial colonies of each strains were inoculated in tryptic soy broth (TSB, Becton Dickinson, Difco, France) for re-growth at 37 °C for 4-6 h. The bacterial suspensions were adjusted to obtain the bacterial cell density of 1.5×108 CFU/mL and further diluted 1:200 in sterile normal saline solution (0.85% NaCl).

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Antimicrobial activity of YRSP was measured by determination of the minimum inhibitory concentration (MIC) of the recipe that needed to inhibit the growth of bacteria was evaluated by broth microdilution method according to CLSI (2018). An aliquot of 100 µL of the ethanolic extract of YRSP was added and diluted by two–fold serial dilution to obtain final concentration ranging from 4–2048 µg/mL, followed by 100 µL of the bacterial suspensions (106 CFU/mL). Ciprofloxacin and series concentration of DMSO ranging from 10-0.02% were used as a standard drug and control, respectively. The cultured plates were incubated at 37 °C for 18 h. Minimum bactericidal concentration (MBC) was determined subsequently to the MIC assays. An aliquot of 10 µL from the wells with MICs was seeded on TSA and incubation at 37 °C for 24 h. The appearance of bacterial colony on the agar plate was evaluated for the MBC determination. The experiment was carried out in triplicate.

LPS-stimulated RAW264.7 cells for NO production

Anti-inflammatory activity of E-YRSP was assessed by determination of NO levels in the supernatant of LPS-stimulated RAW264.7 cells as previously described with partial modification(18). Briefly, RAW 264.7 cells were cultured in DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin at 37°C in 5% CO2. Cells were seeded on 96-well plates at a density of 2 × 105 cells/mL and cultured at 37°C with 5% CO2 for 48 h. The cells were stimulated with LPS (1 µg/mL) and then treated with E-YRSP at various concentrations (0.1, 1, 10, 100, 1,000, and 10,000 µg/mL) at 37 °C with 5% CO2 for 24 h. Triamcinolone acetonide was used as a standard control drug for this experiment. After incubation, cell culture supernatants were collected and the NO level was determined by the Griess reagent method. An absorbance was measure at 550 nm using a microplate reader. The result was indicated as the percentage of nitric oxide inhibition (18).

Cell viability assay

The viability of human dermal fibroblast cells (HDF) was determined using sulforhodamine B (SRB) assay according to previous report (19). Cells (1.9×104 cells/mL) were seeded on 96-well plates containing DMEM supplemented with 10% heat-inactivated FBS and 1% penicillin/streptomycin with or without E-YRSP at different concentrations (0. 1, 1, 10, 100, and 1,000 µg/mL) for 72 h. After incubation, the medium was removed and cells were fixed with 10% trichloroloacetic acid at 4ºC for an hour. After permeabilization, 0.057% SRB (100 µL) was added. After 30 min of incubation, cells were washed with 1% (v/v) acetic acid to remove unbound dye and then allowed the plate to air-dry at room temperature. For detection process, Tris base solution (pH 10.5) was added to each well and the plate was placed in an incubator shaker for 10 min to solubilize the protein-bound dye. The absorbance was measure at 510 nm using a microplate reader and the result was express as % cell viability.

Wound healing activity

To estimate the wound healing activity of E-YRSP, HDF cells were seeded on 24-well plates at a concentration of 3x105 cells/mL, and incubated at 37°C in a humidified atmosphere containing 5% CO2. A linear wound (1 mm) was subsequently created in the cell monolayer using a sterile 200 µL pipette tip. Cells were washed with PBS to remove cell debris and then cells were treated with fresh DEME containing E-YRSP at concentrations of 1,000 µg/mL and incubated for 8, 24, and 48 h at 37 ◦C under 5% CO2 condition. Vitamin C and 10% DMSO were used as a positive and negative control, respectively. At each time, digital pictures were taken and the area of wound was analyzed by Corel draw graphics suite x6 (20).

Statistical analysis

Data were presented as mean ± standard deviation of three independent replicates. Results were statistically analysed using t-test. IBM SPSS version 20 was used to measure the statistical significance which considered at $p < 0.05$.

RESULTS

Quantitative analysis of total phenolics and GC-MS

Determination of total phenolic compounds revealed that E-YRSP was composed of high amount of phenolic compounds at 1,388.22 mgGAE/mg (Table 1). Moreover, GC-MS analysis of E-YRSP indicated the presence of 97 constituents. The retention time (RT), molecular formula, molecular weight (MW), and match score were demonstrated in Table S1. Almost constituents in E-YRSP were identified as phenolic group including catechin, gallocatechin-4beta-ol, rutin, quercetin 3-galactoside, epigallocatechin 3-O-p-coumarate, demethoxycurcumin, luteolin, curcumin, as well as mangostin.

Anti-oxidant activity of E-YRSP

Anti-oxidant activity of E-YRSP was evaluated by DPPH, ABTS, and NO scavenging assays. Results were expressed as IC50 which shown in Table 1. E-YRSP possessed a potent anti-oxidant activity against DPPH, ABTS, and NO radicals which had IC50 value at 15.15±0.06, 6.11±0.02, and 20.341±0.16 µg/mL, respectively.

Anti-bacterial activity of E-YRSP

Anti-bacterial activity of E-YRSP was evaluated using broth microdilution method. YRSP was shown as an effective antibacterial agent, inhibiting Gram positive bacterial strains including S. aureus, S. epidermidis, MRSA, E. faecalis, and B. subtilis. The minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of the extract against of those strains were ranged from 16-128 µg/mL and 32-512 µg/mL, respectively. While, ciprofloxacin, a standard drug, was found MICs and MBCs against Gram-positive bacteria in the range of 0.125-0.5 and 0.25-2 µg/mL, respectively. In Gram-negative bacteria, YRSP was not possessed satisfying inhibitory effects on the standard strains of E. coli, A. baumannii, K. pneumoniae, and P. aeruginosa as shown in Table 2.

Anti-inflammatory activity of E-YRSP

It is well known that excessive production of NO can cause tissue damage, leading to delayed wound healing process. Therefore, inhibition of NO production is one of an important way in the regulation of inflammatory response and wound healing process. The effects of E-YRSP on the inhibition of LPS-stimulated NO production on macrophage RAW264.7 cells were indicated in Fig. 1. It demonstrated that E-YRSP (100 µg/mL) could suppressed NO release with percent inhibition of 26.96±0.79, while triamcinolone acetonide (100 µg/mL), a standard drug, appeared the higher effect with (45.31±5.21% %).

In vitro wound healing activity of E-YRSP

To evaluate wound healing activity of E-YRSP, in vitro scratch wound healing assay was performed. Cytotoxicity of E-YRSP was set before starting the scratch wound healing experiment on HDF cells. It indicated that there were no toxic effects for HDF cells in all tested concentration (0.1-1,000 µg/mL). Interestingly, treatment of E-YRSP at 0.1-1,000 µg/mL appeared to promote HDF cell proliferation comparing to vitamin C, a positive control (Fig. 2). Based on the previous experiment, the concentration of E-YRSP at 1,000 µg/mL was selected for scratch wound healing assay. A result indicated that E-YRSP treated HDF cells resulted in the promotion of cell migration by 24 h of incubation, while there was slightly changed in untreated cells. By 48 h of incubation, cells treated with E-YRSP (50%) showed a higher rate of wound healing as compared to the control group (41.66%) as shown in Fig. 3.

DISCUSSION

Wound healing process is an important part of human body to response to the injury. It is composed of four phases of hemostasis, inflammation, proliferation, and remodelling which must occur in the proper time (1). However, inappropriate response of each phase can lead to delayed wound healing process. Normally, chronic wounds always face with the aggressive inflammatory response which could not allow wounds to pass to the next phase of the healing process (1-2). There are several risk factors affecting the healing response, including local infections, excessive inflammatory mediators, as well as oxidative stress. Therefore, successive wound therapies always need the combination of treatments including topical antibiotics, wound debridement, as well as wound dressing to overcome these causing factors (4, 21). Currently, plant-based materials have been reported as a beneficial agent in wound care and management. YRSP is a traditional Thai herbal recipe that has been used for wound treatment for a long time. It is composed of six medicinal plants including, rhizome of Curcuma longa, seed of Areca catechu L., peel of Garcinia mangostana L., rhizome of Zingiber montanum (Koenig), resins of Uncaria gambier Roxb., and seed of Oryza sativa L. Even it has been widely applied in traditional medicine for wound treatment. However, there are no scientific evidences supporting its traditional use. Interestingly, this study has firstly proved that YRSP possessed competent wound healing activity as well as other biological properties associated with wound healing such as anti-oxidant, anti-inflammatory, and anti-bacterial properties. Previously, there were numerous scientific researches that have been proved the ethnotherapeutic efficacies of each plant in YRSP for anti-inflammation, anti-oxidation, anti-infections, as well as wound healing activities (22-25).

An anti-bacterial activity is also considered as one of the risk factors correlated with delayed wound healing process by provoking an inflammatory response. It led to an excessive production of inflammatory mediators and free radicals at the wound area. Agents targeting to decrease infections as well as to inhibit inflammation are necessary for wound treatment. Our current result intimately revealed that E-YRSP possessed anti-bacterial activity against only Gram-positive bacteria. Moreover, it was found to inhibit inflammation with IC50 up to 1,000 µg/mL and oxidation with IC50 ranged from 6-20 µg/mL. Curcuma longa, one of the herbs in YRSP, has been reported an antibacterial property with MIC value at 3,125 µg/mL against S. aureus and E. faecalis, Gram-positive bacterial stains. Besides, it was found to possess remarkable anti-oxidant activity against DPPH radicals with IC50 at 140.84 µg/mL (23). Apart from this, pericarp of Garcinia mangostana extracts also displayed anti-DPPH radicals with IC50 around 60 µg/ml (24) and strong anti-bacterial activity with MIC value at 100, 400, and 24.04 µg/mL against S. aureus ATCC 25923, MRSA, and S. epidermidis, respectively (22, 26). Zingiber montanum (27), Uncaria gambier (25, 28),

Areca catechu (9, 29) and Oryza sativa (30) have also been represented for anti-infections, anti-inflammation, as well as anti-oxidation. Noticeably, Curcuma longa as well as Areca catechu, have been investigated as wound healing agents by accelerated the process of epithelialization and wound contraction in animal wound model (31-32). Compared to the previous reports, it was interestingly indicated that the combination of these herbs as E-YRSP led to synergistic effects on antibacterial and anti-oxidant activities. It was noted that activities of E-YRSP on anti-infection, anti-oxidation, and anti-inflammation markedly resulted in a competent wound healing efficacy.

Phenolic compounds were abundantly found in E-YRSP as a large group of phytochemical constituents including catechin, rutin, demethoxycurcumin, luteolin, curcumin, as well as mangostin which have been extensively proved as a bioactive agent for wound healing promotion, anti-inflammation, anti-oxidation, and anti-infection in both in vitro and in vivo studies (33-36). Therefore, the efficiency of E-YRSP on anti-inflammation, anti-oxidation, anti-bacteria, and wound healing promotion might result from the biological activities of each plant in the recipe and their bioactive constituents. It is proved that YRSP could be a valuable agent for wound management in clinical practice.

CONCLUSIONS

This study has confirmed that YRSP, a traditional Thai herbal recipe for wound treatment, obviously possessed strong wound healing activity as well as other biological properties associated with wound healing including, anti-oxidation, anti-inflammation, and anti-bacteria. Therefore, it is worthwhile to further study on its mechanisms on wound healing process and also to develop as an alternative treatment for wound treatment.

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COMPETING INTERESTS

No potential conflict of interest was reported by the authors.

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Table 1: Effects of the ethanolic extracts from of Ya-RakSa-Phlae (E-YRSP) recipe on anti-oxidant activities.

Samples	Total phenolics (mgGAE/mg)	Anti-oxidant activity; IC50 (µg/ml)		
		DPPH	ABTS+	NO
E-YRSP	1,388.22	15.15±0.06	6.11±0.02	20.341±0.16
Trolox	NA	8.47±0.13	4.22±0.02	16.29±0.30

Table 2: Antibacterial activities of the ethanolic extracts of Ya-RakSa-Phlae (E-YRSP) recipe evaluated and ciprofloxacin susceptibility testing by microdilution method.

Bacterial strains	MICs/MBCs (µg/mL) E-YRSP	Ciprofloxacin
Staphylococcus aureus ATCC 25923	32/128	0.25/1
Staphylococcus epidermidis ATCC 35984	16/32	0.125/0.5
Methicillin-resistant S. aureus (MRSA)	128/512	0.5/2
Bacillus subtilis	128/256	0.125/0.25
Escherichia coli ATCC 25922	>2048/NA	0.016(S)/0.031
Escherichia coli O157-H7	>2048/NA	0.062(S)/0.125
Enterococcus faecalis ATCC 29212	128/256	0.125/0.5
Acinetobacter baumannii ATCC 19606	1024/>2048	0.5(1)/1
Klebsiella pneumoniae ATCC 700603	>2048/NA	0.031(S)/0.062
Pseudomonas aeruginosa ATCC 27853	>2048/NA	0.062(S)/0.062

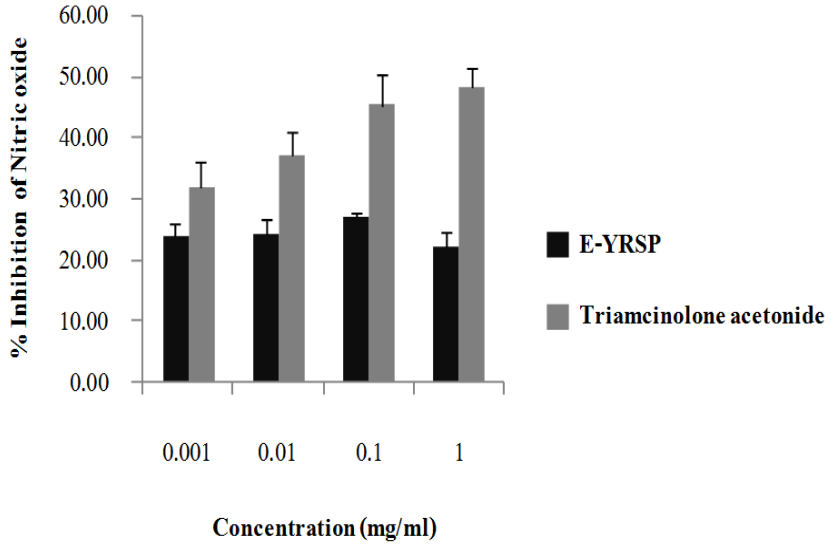

Figure 1: Anti- inflammatory activity of E-YRSP in LPS-stimulated RAW 264.7 macrophages.

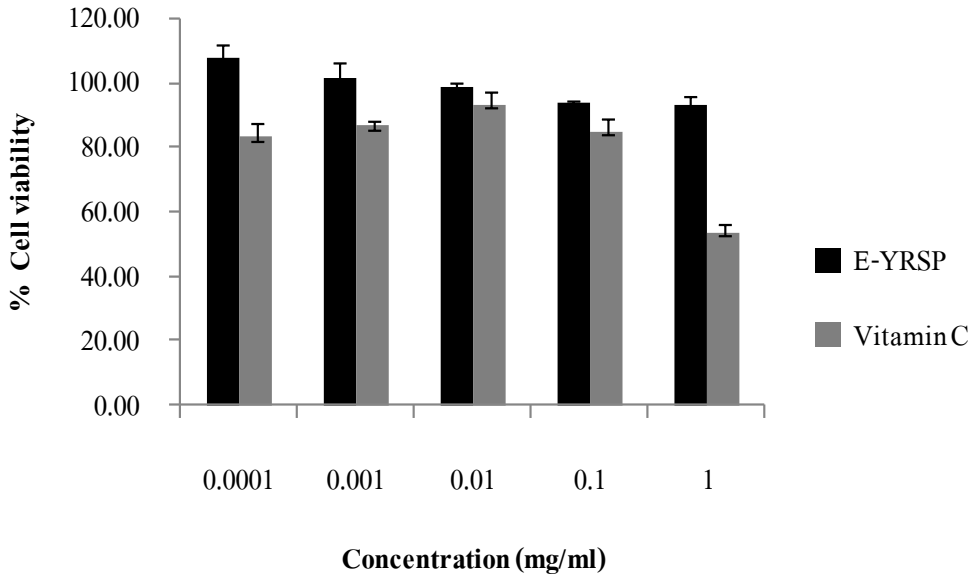

Figure 2: The effect of E-YRSP on human dermal fibroblast cell viability.

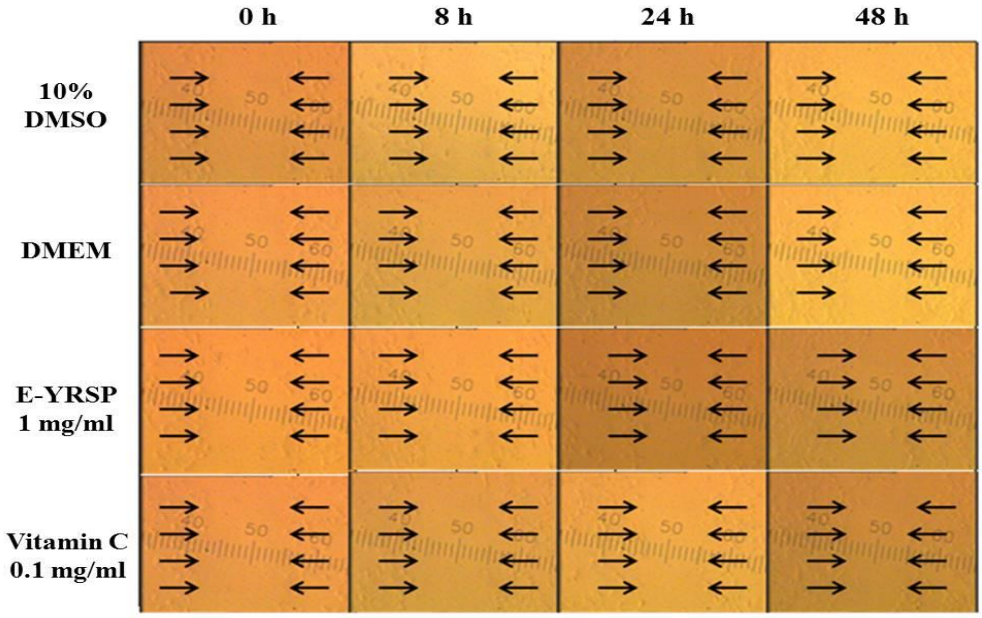

Figure 3: Effects of E-YRSP on the promotion of human dermal fibroblast migration observed using the scratch assay.

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae.

RT	Name	MW	Formula	Math score
1.9166	Quinic acid	192.06	C7H12O6	99.77
2.191	Pimelic acid	160.07	C7H12O4	84.86
2.722	3-methyl-adipic acid	160.07	C7H12O4	87.03
5.534	Procyanidin B2	578.14	C30H26O12	98.83
5.982	Catechin	290.07	C15 H14 O6	98.94
6.023	6"-p-Coumaroylprunin	580.15	C30 H28 O12	47.61
6.09	Cynarotrioside	756.21	C33 H40 O19	99.4
6.269	Vitexin4'-O-glucoside-2"-O-rhamnoside	740.21	C15 H14 O8	99.3
6.336	Gallocatechin-4beta-ol	322.06	C15 H14 O8	99.04
6.42	trans-Grandmarin	292.09	C15 H16 O6	99.28
6.56	Rutin	610.15	C27 H30 O16	93.92

6.838	Quercetin 3-galactoside	464.09	C21 H20 O12	99.54
7.075	Kaempferol3-[2"-(6"'-coumaroylglucosyl) -rhamnoside] 7-glucoside	902.24	C42 H46 O22	99.01
7.152	Rutilantinone	428.11	C22 H20 O9	92.98
7.337	Salfredin B11	232.07	C13 H12 O4	97.99

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae. (Continue)

RT	Name	MW	Formula	Math score
7.532	Kaempferol3-rhamnosyl-(1->3) (4"'-p-coumarylrhamnosyl) (1->6)-glucoside	886.25	C42 H46 O21	97.17
7.612	Streptonigrin	506.15	C25 H22 N4 O8	80.63
7.727	Epigallocatechin3-O-p-coumarate	452.11	C24 H20 O9	98.51
8.008	6-Prenylisocaviunin	442.16	C15 H26 O9	99.25
8.031	Rutilantinone	428.11	C22 H20 O9	99.43
8.098	Dihydroisorhamnetin	318.07	C16 H14 O7	99.26
8.273	Ethyl 6,7-dimethoxy-4-oxo-2,3-dihydro-1H-naphthalene-2-carboxylate	278.12	C15 H18 O5	99.27
8.449	Salfredin B11	232.07	C13 H12 O4	98.9
8.673	Dukunolide A	482.16	C26 H26 O9	95.69
8.763	Epigallocatechin 3-O-cinnamate	436.12	C24 H20 O8	95.74
8.897	Silymonin	466.13	C25 H22 O9	96.38
9.306	8-Acetoxy-4'- methoxypinoresinol	430.16	C23 H26 O8	99.42
9.405	Gibberellin A51-catabolite	330.15	C19 H22 O5	99.52

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae. (Continue)

RT	Name	MW	Formula	Math score
9.67	Urolithin D	260.03	C13 H8 O6	99.34
9.692	Silymonin	466.13	C25 H22 O9	94.8
10.079	4-Benzyloxy-2'-hydroxy-3', 4', 5', 6'-tetramethoxychalcone	450.17	C26 H26 O7	99.22
10.38	Kuwanon Y	582.19	C34 H30 O9	72.74
10.616	Hieracin	302.04	C15 H10 O7	99.92
10.884	Leucadenone D	540.21	C33 H32 O7	98.94
11.096	7-[(6-Hydroxy-3,7- dimethyl-2, 7-octadienyl) oxy]-2H-1-benzopyran-2-one	314.15	C19 H22 O4	99.3
11.259	S-Adenosylhomocysteine	384.12	C14 H20 N6 O5 S	83.10
11.978	Neobanone	382.10	C21 H18 O7	98.03
12.298	Macaflavone II	434.17	C26 H16 O6	98.03
12.8	Warfarin	308.10	C19 H16 O4	98.07
12.812	Demethoxycurcumin	338.12	C20 H18 O5	99.14
13.026	Luteolin	286.05	C15 H10 O6	99.85
13.154	2,8-Dihydroxy-3,9,10-trimethoxypterocarpan	346.11	C18 H18 O7	99.35
13.288	2-Isoprenylemodin	338.12	C20 H18 O5	99.53
13.288	Physalin M	512.20	C28 H32 O9	98.88
13.551	Ponganone VIII	426.17	C24 H26 O7	99.27

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae. (Continue)

RT	Name	MW	Formula	Math score
13.864	5'-Demethoxydeoxy-podophyllotoxin	368.13	C21 H20 O6	95.67
14.004	Laxiflorin	414.17	C23 H26 O7	98.49
14.196	4beta-Hydroxyobovata- chromene	370.14	C21 H22 O6	99.42
14.49	Absintholide	526.26	C30 H38 O8	98.95
14.631	Dihydroamorphigenin	412.15	C23 H24 O7	99.26
14.842	Cycloartocarpin A	434.17	C26 H26 O6	97.85
15.347	Pseudolaric Acid B	432.18	C23 H28 O8	98.71
15.609	PHYSALIN B	510.19	C28 H30 O9	99.48
15.861	6-Acetylpicropolin	460.17	C25 H28 O9	99.2
16.235	Rhodomyrtoxin B	428.18	C24 H28 O7	97.46
16.644	Isophysalin B	510.19	C28 H30 O9	93.63
17.44	5-Hydroxywarfarin	324.10	C19 H16 O5	99.40
17.505	5'-O-Methylmelledonal	446.19	C24 H23 O8	97.86
17.651	2-(4-Ethoxyphenyl)-5,6,7,8-tetramethoxy-4H-1-benzopyran-4-one	386.14	C21 H22 O7	97.49
17.779	Artoindonesianin B	468.18	C26 H28 O8	94.84
18.099	8-Acetoxy-4'- methoxypinoresinol	430.16	C23 H26 O8	99.48

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae. (Continue)

RT	Name	MW	Formula	Math score
18.099	8-Acetoxy-4'- methoxypinoresinol	430.16	C23 H26 O8	99.48
18.223	3,5,7-Trihydroxy-6- methoxy-4'-prenyloxyflavone	384.12	C21 H20 O7	99.44
18.329	Asarinin (-)	354.11	C20 H18 O6	99.57
18.485	Mangostenol	426.17	C24 H26 O7	98.19
18.813	Tomentosanol E	508.25	C30 H36 O7	98.49
19.061	Kushenol T	426.20	C25 H30 O6	98.44
19.687	Tanariflavanone A	508.25	C30 H36 O7	99.85
19.978	Acrovestone	554.29	C32 H42 O8	99.69
20.588	Bisdemethoxycurcumin	308.11	C19 H16 O4	91.76
20.755	(+-)-5-Deoxykievitone	340.13	C20 H20 O5	98.35
20.979	Demethoxycurcumin	338.12	C20 H18 O5	98.16
21.199	5-Hydroxy-4,6,4'-trimethoxyaurone	328.09	C18 H16 O6	99.63
21.343	Curcumin	368.13	C21 H20 O6	88.64
21.493	Wortmannin	428.15	C23 H24 O8	96.88
21.573	Glycinoeclepin C	514.26	C29 H38 O8	88.64
21.679	Idarubicinol aglycone	370.11	C20 H28 O7	96.63
21.695	8-Acetoxy-4'-methoxypinoresinol	430.16	C23 H26 O8	99.11

Table S1: Bioactive compounds identified in the ethanol extract of Ya-RakSa-Phlae. (Continue)

RT	Name	MW	Formula	Math score
21.831	Exiguaflavanone J	508.25	C30 H36 O7	97.53
21.941	Laxiflorin	414.17	C23 H26 O7	97.6
22.053	Kanzonol M	398.17	C23 H26 O6	98.85
22.209	Dihydrorotenone	396.16	C23 H24 O6	81.12
22.449	Laxiflorin	414.17	C23 H26 O7	94.48
22.541	Mangostin	410.17	C24 H26 O6	82.1
22.692	2',4',6',3,4-Pentahydroxy-3'-geranyl-5-prenyldihydrochalcone	494.27	C30 H38 O6	95.24
22.897	Rhodomyrtoxin	428.19	C24 H28 O7	91.26
23.475	Dihydroamorphigenin	412.15	C23 H24 O7	81.57
23.715	(+)-Tephrorin A	426.17	C24 H26 O7	97.79
23.788	Dihydroamorphigenin	412.15	C23 H24 O7	86.3
23.916	Semilicoisoflavone B	352.10	C20 H16 O6	99.18
24.156	1,7-Dideacetoxy-1, 7-Dioxokhivorin	498.23	C28 H34 O8	99.54
24.172	Scarlet Red	380.16	C24 H20 N4 O	63.75
24.351	dimethoxy Curcumin	396.16	C23 H24 O6	92.14
24.52	Rhodomyrtoxin B	428.18	C24 H28 O7	98.51
24.695	Dulciol C	482.23	C28 H34 O7	98.88
29.788	PI(O-16:0/18:2(9Z,12Z))	820.54	C43 H81 O12 P	54.43
29.829	1,2-Di-O-palmitoyl-3-O-(6-sulfoquinovopyranosyl) glycerol	794.52	C41 H78 O12 S	92.64
30.062	MGDG (18:3(9Z,12Z,15Z) 18:3(9Z,12Z,15Z	774.53	C45 H74 O10	84.40