Original Article

Assessment of *in vitro* Antioxidant Activities and Quantification of Total Phenolic and Flavonoid Contents in Extracts from The Thai Traditional Remedy "Ruean-Khi-Nok" and its Plant Constituents

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Abstract

Introduction: Psoriasis is an immune-mediated inflammatory skin disease. The pathogenesis of psoriasis has been associated with an increase of oxidative stress. Therefore, natural antioxidant compounds (e.g., phenolics and flavonoids) might be beneficially used as an adjuvant. Ruean-Khi-Nok (RKN) remedy is a Thai traditional preparation used to treat psoriatic skin, consisting of nine plants in an equal proportion.

Objectives: To investigate the antioxidant capability of the RKN remedy and its components using three chemical-based assays, as well as to determine phenolic and flavonoid contents.

Methods: The 95% and 40% ethanolic extracts of RKN remedy and its plant components were investigated for *in vitro* antioxidant activities using DPPH and ABTS radical scavenging assay, as well as FRAP assay. The total phenolic and flavonoid contents in extracts were quantified using the Folin-Ciocalteu's method and the aluminum chloride colorimetric method, respectively.

Results: The results exhibited potential antioxidant activity of the 95% ethanolic extract of RNK remedy in the DPPH, ABTS and FRAP assay. In addition, it also contained high phenolic and flavonoid contents. Among individual plants, *Piper wallichii* extracts displayed outstanding antioxidant capability compared to the others.

Conclusions: The RKN remedy is therefore highly promising antioxidant and might support the traditional use of RKN remedy for treatment of psoriatic skin. However, RKN should be investigated further for psoriasis treatment.

Keywords: Ruean-Khi-Nok remedy, Antioxidant activity, Total phenolic content, Total flavonoid content

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Introduction

Psoriasis is a chronic immune-mediated inflammatory skin disease, affecting about 3% of the world's population.[1] In addition, Psoriatic skin can have negative impact on patients' quality of life.[2] Recently, it has been found that psoriasis pathogenesis may be linked to oxidative stress, an imbalance between oxidants and antioxidants.[3] This condition causes an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to cellular damage or dysfunction through various mechanisms, such as the secretion of pro-inflammatory cytokines and hyperproliferation of keratinocytes.[1,4]

In recent years, several studies have emerged involving phenolics and flavonoids, plant-derived natural compounds, as a potential recovery tool for treatment of psoriasis owing to their powerful antioxidant properties.[5-7] Ruean-Khi-Nok (RKN) remedy, a topical preparation in the National Thai Traditional Medicine Formulary (Special Edition), has been used for the treatment of psoriatic skin.[8] It consists of nine plant species in equal proportions: the leaves of *Casearia grewiifolia* Vent. (CG), the leaves of *Crateva religiosa* G. Forst. (CR), the leaves of *Crateva adansonii* DC. (CA), the leaves of *Piper wallichii* (Miq.) Hand. -Mazz. (PW), the leaves of *Datura metel* L. var. *metel* (DM), the leaves of *Persicaria chinensis* (PC) (L.) H. Gross, the aerial part of *Pouzolzia zeylanica* (L.) Benn. (PZ), the aerial part of *Gonostegia pentandra* (Roxb.) Miq. (GP), and the rhizome of *Alpinia galanga* (L.) Willd. (AG). Previous studies suggested that *A. galanga* extract may exert its anti-psoriatic effect by modulating NF-κB signaling biomarkers.[9] In addition, withanolides, the active compounds isolated from *D. metel* leaves also showed promise in treatment of psoriasis by reducing inflammatory cytokines, as well as lowering HIF-1α and VEGF expression in angiogenesis.[10] Furthermore, several studies have reported antioxidant activity of some individual plant components using different assays, including DPPH and ABTS radical scavenging assay.[11-16] Despite this data, there is no scientific research available on antioxidant activity of RKN remedy as well as total phenolic and flavonoid contents. Hence, we aimed to investigate the antioxidant activities of Ruean-Khi-Nok remedy and its plant components using three anti-oxidant methods, DPPH radical scavenging assay, ABTS radical scavenging assay and Ferric reducing antioxidant power (FRAP) assay, as well as total phenolic and total flavonoid contents.

Methods

2.1 Plant materials

The leaves of *C.grewiifolia* Vent. (CG), the leaves of *C. religiosa* G. Forst. (CR), the leaves of *C. adansonii* DC. (CA), the leaves of *P. wallichii* (Miq.) Hand.-Mazz. (PW), the leaves of *D. metel* L. var. *metel* (DM), the leaves of *P. chinensis* (PC) (L.) H. Gross, the aerial part of *P. zeylanica* (L.) Benn. (PZ), the aerial part of *G. pentandra* (Roxb.) Miq. (GP), and the rhizome of *A. galanga* (L.) Willd. (AG) were purchased from a local herbal shop, Bangkok, Thailand. The voucher specimens were identified and deposited at the herbarium of Thai Traditional Medicine Research Institute, Department of Thai Traditional and Alternative Medicine, Bangkok, Thailand, which are TTM 1000658, TTM 1000659, TTM 1000660, TTM 1000663, TTM 0005448, TTM 1000664, TTM 0005449, TTM 1000662 and TTM 0005447, respectively.

2.2 Chemicals and reagents

Butylated hydroxytoluene (BHT) and 1,1-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Fluka (Germany). Aluminum chloride, gallic acid and sodium nitrite were purchased from TCI, Japan. Absolute ethanol, hydrochloric acid (37%) and glacial acetic acid were purchased from RCI Labscan, Thailand. Folin-Ciocalteu's reagent, sodium carbonate, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium acetate, 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ), sodium acetate trihydrate, ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and all the other chemicals not specifically mentioned were obtained from Sigma Aldrich, Germany.

2.3 Preparation of Ruean-Khi-Nok (RNK) remedy and its plant component extracts

All plant materials were cleaned, dried and ground into coarse powder. For the preparation of RKN remedy, according to traditional use, each

plant component was used in equal portions.[8] The RKN remedy was then mixed with spirit (about 40% alcohol) before being used. Thus, the extracts of RKN remedy and its plant constituent were obtained by maceration method with 40% and 95% ethanol. To do so, the RKN remedy and its plant components were macerated with 40% and 95% ethanol for 3 days. The extracts were filtrated through Whatman No.1 filter paper and then concentrated under reduced pressure using rotary evaporator. The maceration process was repeated twice (a total of 3 times). Finally, the extracts were dried in a vacuum dryer. All extracts were stored at -20 °C for further experiments.

2.4 *In vitro* antioxidant activity

2.4.1 DPPH radical scavenging assay

The DPPH radical scavenging activity of the ethanolic extracts of the RKN remedy and its components was evaluated according to the modified method described by Yamasaki and colleagues.[17] In brief, the tested samples were firstly prepared with different concentrations (final concentration of 6.25 to 200 µg/mL). DPPH (1.2 mg) was dissolved in absolute ethanol (50 mL) to make up a DPPH reaction solution (6×10^{-5} M). One hundred microliters of the tested samples were added to 96-well plate. Subsequently, DPPH solution (100 µL) was then added to the samples, followed by incubation at room temperature for 30 min (protected from light). The absorbance was measured at a wavelength of 520 nm using a microplate reader. BHT was used as a positive control. The results were expressed as the percent inhibition of absorbance of DPPH and half-maximal effective concentrations (EC_{50}).

2.4.2 ABTS radical scavenging assay

The ABTS radical scavenging activity of the ethanolic extracts of the RKN remedy and its components was determined according to the method described by Re and colleagues[18] with some modifications. Firstly, ABTS solution (7.2 mM) was mixed with potassium persulfate solution (2.45 mM) in an equal portion and allowed them to react overnight (12 -16 hours) at low temperatures (4°C) in the dark. The resulting blue-green colored $ABTS^{\bullet+}$ radical cation solution was diluted with deionized water to obtain the absorbance between 0.680 -0.720 at wavelength of 734 nm before using in the assay. Twenty microliters of the tested samples at different concentrations (final concentration ranging from 1.5625 to 200 µg/mL) were added to 96-well plate. Subsequently, $ABTS^{\bullet+}$ radical cation solution (180 µL) was then added to the samples, followed by incubation at room temperature for 6 min (protected from light). Trolox and BHT were used as positive control. The results were expressed as the percent inhibition of absorbance of ABTS and EC_{50}.

2.4.3 Ferric reducing antioxidant power (FRAP) assay

The antioxidant power of the ethanolic extracts of the RKN remedy and its components was assessed using ferric reducing ability according to the method described by Benzie and Strain.[19] In brief, the FRAP reagent was firstly prepared by mixing 300 mM acetate buffer (pH 3.6) with 10 mM TPTZ solution (in 40 mM HCl), and 20 mM ferric chloride solution at the ratio of 10:1:1 (v/v/v). A standard calibration curve of Trolox and $FeSO_4$ was prepared with different concentration ranging from 6.25 to 300 and 6.25 to 800 µg/mL, respectively. Twenty microliters aliquot of the tested samples solution was added in 96-well plate and mixed with 180 µL of the FRAP reagent (pre-warmed at 37 °C). The mixture was allowed to react at room temperature for 8 min before being measured at a wavelength of 593 nm using a microplate reader. The antioxidant power was calculated by correlating the absorbance of each sample with the standard calibration curve of Trolox and $FeSO_4$. The results were expressed as Trolox equivalent antioxidant capacity (TEAC value; mg TE/g extract) and ferric reducing antioxidant power (FRAP value; mg Fe^{2+}/g extract).

2.5 Total phenolic content

Total phenolic content of the ethanolic extracts of RKN remedy and its components was measured using the Folin-Ciocalteu's method as previously described.[20,21] Briefly, A standard calibration curve of gallic acid was prepared at 6 concentrations (6.25, 12.5, 25, 50, 75 and 100 µg/mL). Twenty microliters aliquot of the tested samples was added in 96-well plate and mixed with Folin-Ciocalteu's reagent (100 µL). Subsequently, sodium carbonate solution (80 µL) was added to the samples, followed by incubation for 30 min at room temperature. The absorbance was determined at a wavelength of 765 nm using a microplate reader. The total phenolic content was calculated by correlating the absorbance of each sample with the standard curve of gallic acid

and expressed as milligrams of gallic acid equivalent per 1 gram of extract (mg GAE/g extract).

2.6 Total flavonoid content

Total flavonoid content of the ethanolic extracts of RKN remedy and its components was determined using the aluminum chloride colorimetric method as previously described.[22] To do so, catechin was firstly prepared as a standard flavonoid solution in absolute ethanol (concentration ranging from 6.25 to 400 µg/mL). Five hundred microliters of the tested samples were mixed with 75 µL of sodium nitrite (5% w/v), 150 µL of aluminum chloride (10% w/v), 500 µL of sodium hydroxide (1M) and 275 µL of distilled water, respectively, in a centrifuge tube. One hundred microliters of the mixture were then transferred into 96 well-plate and incubated at room temperature for 30 minutes. The absorbance was measured at a wavelength of 510 nm using a microplate reader. The total flavonoid content was calculated by correlating the absorbance of each sample with the standard curve of catechin. The results were expressed as milligrams of catechin equivalent per 1 gram of extract (mg CE/g extract).

2.7 Statistical analysis

All experiments were expressed as mean ± standard error of mean (SEM) from at least four separate experiments. Statistically significant differences in radical scavenging activity among plant extracts and positive control were performed using one-way analysis of variance analysis (ANOVA) followed by Dunnett's multiple comparison test. Differences were considered statistically significant for *p-values* lower than 0.05.

Results

3.1 *In vitro* antioxidant activity

3.1.1 DPPH radical scavenging assay

The DPPH radical scavenging activity of the ethanolic extracts of RKN remedy and its plant components are shown in Table 1. No significant differences ($p > 0.05$) were observed between the activity of RKNE95 extract and BHT (EC_{50}: 15.00 ± 0.33 µg/mL for RKNE95 and 15.38 ± 0.22 µg/mL for BHT), while the RKNE40 extract (EC_{50}: 183.49 ± 1.28 µg/mL) exhibited very low activity. Among all the plants components extracted with 95% ethanol, the PWE95 extract (EC_{50}: 3.44 ± 0.10 µg/mL) possessed the highest activity and significantly better than BHT, while the others possessed moderate ability. In contrast, most plants extracted with 40% ethanol exhibited low DPPH radical scavenging activity (EC_{50} >200 µg/mL), except PWE40 (EC_{50}: 22.63 ± 0.29 µg/mL) and PCE40 (EC_{50}: 38.14 ± 1.15 µg/mL) extracts.

3.1.2 ABTS radical scavenging assay

The ABTS radical scavenging activity of the ethanolic extracts of RKN remedy and its plant components are shown in Table 1. It was evident that RKNE95 extract (EC_{50}: 20.32 ± 0.30 µg/mL) had 2-fold higher ABTS scavenging activity than the RKNE40 extract (EC_{50}: 43.47 ± 0.94 µg/mL). Among all the ingredients extracts, the PWE95, PWE40 and PCE40 extracts exerted potent ABTS radical scavenging activity (EC_{50}: 6.17 ± 0.19, 8.29 ± 0.11 and 8.87 ± 0.20 µg/mL, respectively), but slightly less activity as compared to BHT (EC_{50}: 2.53 ± 0.02 µg/mL) and Trolox (EC_{50}: 4.16 ± 0.08 µg/mL). Meanwhile, other component plant extracts possessed moderate ABTS radical scavenging activity in accordance with the RKN extracts (EC_{50} ranging from 13.61 to 39.92 µg/mL), except the GPE40, and both extracts of PZ and DM (EC_{50} > 50 µg/mL).

3.1.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP of the ethanolic extracts of RKN remedy and its plant components are shown in Table 2. The results found that the TEAC and FRAP values of the RKNE95 extract (126.48 ± 1.42 mg TE/g extract and 272.14 ± 6.43 mg Fe^{2+}/g extract) were 2.5-fold higher than the RKNE40 extract (51.44 ± 1.96 mg TE/g extract and 112.02 ± 0.93 mg Fe^{2+}/g extract). Among all the component plants extracts, the highest TEAC and FRAP values were found in the PWE95 (419.07 ± 7.24 mg TE/g extract and 902.94 ± 11.44 mg Fe^{2+}/g extract) followed by the PWE40 (268.78 ± 5.72 mg TE/g extract and 582.74 ± 7.98 mg Fe^{2+}/g extract) extracts. In addition, the PCE95, PCE40, CGE40 and CAE40 extracts also exhibited good antioxidant activity compared to PWE40, with values ranging from 224.00 ± 3.20 to 110.92 ± 2.77 mg TE/g extract. However, the others exhibited lower reducing antioxidant power than PWE40.

Table 1 DPPH and ABTS radical scavenging activities of the ethanolic extracts of RKN remedy and its plant components (n = 6)

Sample	Code	EC_{50} (µg/mL)	
		DPPH assay	**ABTS assay**
Ruean-Khi-Nok remedy	RKNE95	15.00 ± 0.33	20.32 ± 0.30[a,b]
	RKNE40	183.49 ± 1.28[a]	43.47 ± 0.94[a,b]
C. grewiifolia Vent.	CGE95	12.39 ± 0.28[a]	23.03 ± 0.21[a,b]
	CGE40	154.13 ± 2.18[a]	13.61 ± 0.54[a,b]
C. religiosa G. Forst.	CRE95	33.02 ± 1.01[a]	35.54 ± 0.49[a,b]
	CRE40	> 200[a]	24.90 ± 0.68[a,b]
C. adansonii DC.	CAE95	26.01 ± 0.88[a]	31.10 ± 0.45[a,b]
	CAE40	176.47 ± 3.26[a]	22.04 ± 0.44[a,b]
P. zeylanica (L.) Benn.	PZE95	69.65 ± 1.38[a]	142.48 ± 2.68[a,b]
	PZE40	> 200[a]	186.49 ± 2.82[a,b]
G. pentandra (Roxb.) Miq.	GPE95	17.29 ± 0.26[a]	39.92 ± 0.29[a,b]
	GPE40	> 200[a]	91.94 ± 1.30[a,b]
A. galanga (L.) Willd.	AGE95	19.87 ± 0.75[a]	21.17 ± 0.86[a,b]
	AGE40	> 200[a]	40.88 ± 1.26[a,b]
P. wallichii (Miq.) Hand.-Mazz.	PWE95	3.44 ± 0.10[a]	6.17 ± 0.19[a,b]
	PWE40	22.63 ± 0.29[a]	8.29 ± 0.11[a,b]
D. metel L. var. *metel*	DME95	37.91 ± 0.94[a]	60.55 ± 1.19[a,b]
	DME40	> 200[a]	80.04 ± 1.00[a,b]
P. chinensis (L.) H. Gross	PCE95	11.08 ± 0.44[a]	17.76 ± 0.18[a,b]
	PCE40	38.14 ± 1.15[a]	8.87 ± 0.20[a,b]
Buthylhydroxytoluene	BHT	15.38 ± 0.22	2.53 ± 0.02
Trolox	Trolox	-	4.16 ± 0.08

Note: Code E95 was 95% ethanolic extract and E40 was 40% ethanolic extract. [a] Significant differences ($p < 0.05$) were observed compared to BHT and [b] Significant differences ($p < 0.05$) were observed compared to BHT and Trolox.

Table 2 The ferric reducing antioxidant power (FRAP) assay of the ethanolic extracts of RKN remedy and its plant components

Sample	Code	TEAC value (mg TE/g extract)	FRAP value (mg Fe^{2+}/g extract)
Ruean-Khi-Nok remedy	RKNE95	126.48 ± 1.42	272.14 ± 6.43
	RKNE40	51.44 ± 1.96	112.02 ± 0.93
C. grewiifolia Vent.	CGE95	66.33 ± 1.05	143.91 ± 3.24
	CGE40	207.28 ± 9.91	443.38 ± 8.79
C. religiosa G. Forst.	CRE95	57.51 ± 1.74	125.02 ± 2.30
	CRE40	89.30 ± 3.84	192.52 ± 3.03
C. adansonii DC.	CAE95	81.88 ± 1.02	177.04 ± 3.52
	CAE40	110.92 ± 2.77	238.77 ± 2.82
P. zeylanica (L.) Benn.	PZE95	19.59 ± 0.63	44.28 ± 0.43
	PZE40	9.12 ± 0.68	21.93 ± 0.65
G. pentandra (Roxb.) Miq.	GPE95	54.38 ± 1.09	118.46 ± 3.54
	GPE40	16.73 ± 0.69	38.17 ± 0.57
A. galanga (L.) Willd.	AGE95	52.61 ± 1.68	114.60 ± 2.48
	AGE40	38.56 ± 0.67	84.78 ± 2.85
P. wallichii (Miq.) Hand.-Mazz.	PWE95	419.07 ± 7.24	902.94 ± 11.44
	PWE40	268.78 ± 5.72	582.74 ± 7.98
D. metel L. var. *metel*	DME95	47.24 ± 0.73	103.29 ± 3.47
	DME40	24.59 ± 1.10	54.90 ± 1.48
P. chinensis (L.) H. Gross	PCE95	116.79 ± 2.71	251.26 ± 1.64
	PCE40	224.00 ± 3.20	479.76 ± 6.18

Note: Code E95 was 95% ethanolic extract and E40 was 40% ethanolic extract.

3.2 Total phenolic content

The content of phenolic compounds in the ethanolic extracts of RKN remedy and its plant components are presented in Figure 1. The RKNE95 extract content of phenolics was 100.39 ± 2.29 mg GAE/g extract, while the RKNE40 extract had 68.77 ± 0.98 mg GAE/g extract. The highest total phenolic content was found in both PWE95 and PWE40 (355.69 ± 4.65 mg GAE/g extract for PWE95 and 341.33 ± 2.10 mg GAE/g extract for PWE40).

3.3 Total flavonoid content

The total flavonoid content in the ethanolic extracts of RKN remedy and its plant components are presented in Figure 2. The results showed that the RKNE95 extract contained 3.8-fold higher total content of flavonoids than the RKNE40 extract (133.54 ± 1.62 mg CE/g extract for RKNE95 and 34.82 ± 0.53 mg CE/g extract for RKNE40). Both PWE95 and PWE40 had the highest flavonoid contents of 493.95 ± 2.69 and 451.62 ± 2.00 mg CE/g extract, respectively.

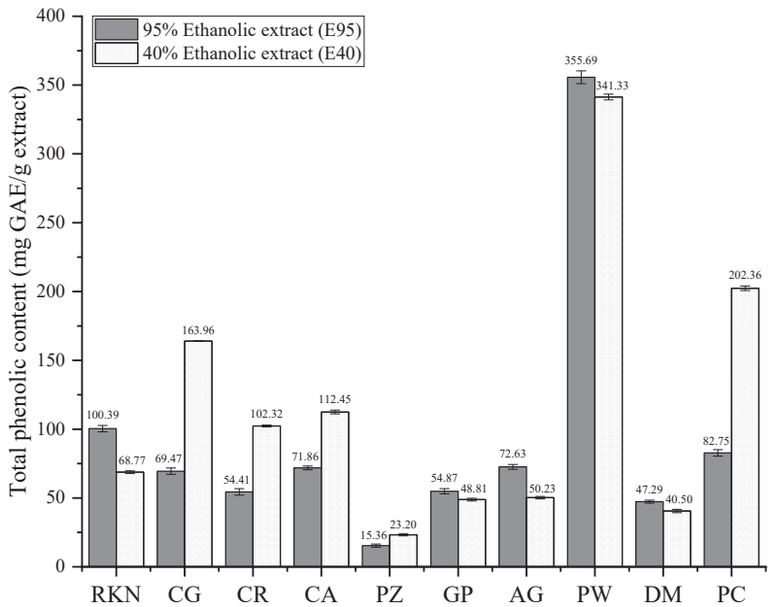

Figure 1 Total phenolic content of the ethanolic extracts of RKN remedy and its plant components (n = 4)

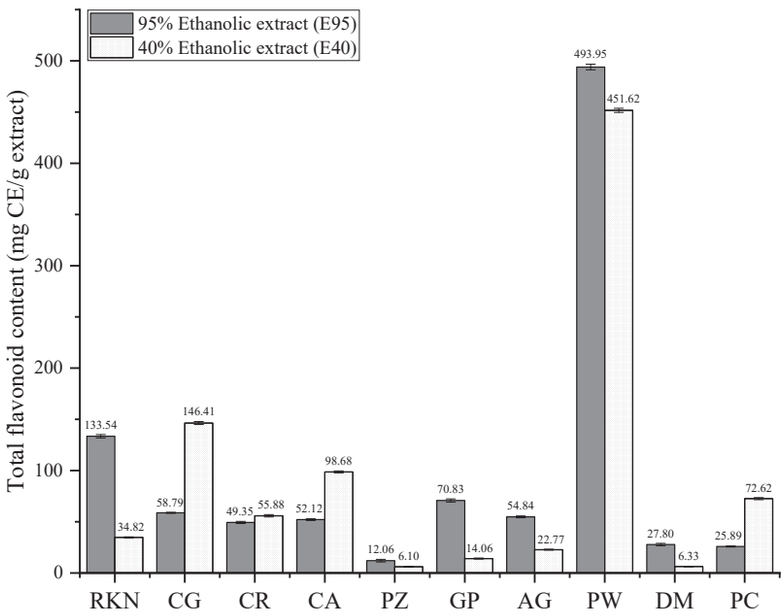

Figure 2 Total flavonoid content of the ethanolic extracts of RKN remedy and its plant components (n = 4)

Discussion

Oxidative stress has been proposed as one of the risk factors involved in the pathogenesis of psoriasis. Therefore, using antioxidant compounds might be a potential adjuvant for treatment of this dermatosis.[3, 23] In this study, we selected several chemical-based antioxidant methods to evaluate the antioxidant activity, as well as to determine total phenolic and flavonoid contents of the RKN remedy and its component plants extracts.

The DPPH radical scavenging assay is one of the most widely used method for screening antioxidant activity of plant extracts due to its simplicity, low cost, and high sensitivity with a rapid reaction.[17] However, the DPPH radical chromogens can only be dissolved in an organic solvent which limits its

application.[24] On the other hand, The ABTS radical scavenging assay is more adaptable for assessing the antioxidant capacity than the DPPH assay because the ABTS cationic radical can be soluble in both organic and aqueous mediums. Therefore, it can be used to screen both lipophilic and hydrophilic tested samples.[25] The FRAP assay is also used to evaluate antioxidant power of a compound based on its capacity to reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}). This method is suitable for screening a wide spectrum of samples, for example biological fluids, organic extracts, foods, and plants.[26]

In this current study, the RNK remedy has been shown to have a promising antioxidant effect in all tested methods, especially for the 95% ethanolic extract, as evidenced by similarly scavenged DPPH and ABTS radicals compared to standard BHT and Trolox. From the FRAP assay, the RKNE95 extract also exhibited a potential antioxidant capacity. Regarding specific plant components, the PW extracts displayed outstanding antioxidant capability. Their activities are similar or even better than the positive control. Additionally, the extracts of PC and CG were shown to be good scavengers against free radicals. The antioxidant activity of several component plants in the RKN remedy have previously been investigated using various experimental models. For example, Tamuly and colleague reported the scavenging activity (DPPH, ABTS and FRAP assay) of the PW (leaves) extracted with methanol and 50% methanol with the EC_{50} value in the range of 48.4 ± 1.83 to 69.7 ± 0.84 µg/mL.[9] Moreover, the methanolic extract of the CG (leaves) was shown to be a potent inhibitor of the ABTS radical with the EC_{50} value of 24.9 µg/mL, consistent with our results.[12]

It is well established that phenolics and flavonoids are classified as free radical scavengers due to their hydroxyl groups bonded to a benzene ring or complex aromatic ring structures.[27] These interesting molecules can interrupt or neutralize the oxidative stress by donating their electrons to free radicals.[28] Our results indicated high content of phenolics and flavonoids in both PWE95 and PWE40 compared to other extracts. In addition, RKNE95 also contained higher phenolics and flavonoids than RKNE40. These findings are in accordance with the results obtained from antioxidant activity assays. Similar outcome was also reported by several research groups. For instance, the fruit of PW extracted with methanol exhibited the highest phenolic content (75.37 ± 1.75 mg GAE/g) compared to other solvent extracts. This methanolic extract also showed the best DPPH and ABTS radical scavenging activity (EC_{50} values of 46.70 ± 0.85 and 45.23 ± 2.02 µg/mL).[13] By contrast, the methanolic extract of DM which contained low phenolic (46.09 ± 0.4 mg GAE/g extract) and flavonoid content (21.71 ± 0.12 mg rutin equivalent/g extract), resulting in low DPPH and ABTS scavenging activities with the IC_{50} values of 180.97 ± 5.49 and 304.63 ± 25.39 µg/mL), respectively.[15]

In conclusion, this study is the first report to reveal the potential antioxidant ability of the RKN remedy, especially when extracted with 95% ethanol. Among its plant components, the PWE95 displayed the highest antioxidant capacity compared to the others. These results are promising and might support the traditional use of RKN remedy for treatment of psoriatic skin. However, further investigations related to the pathogenesis of psoriasis, as well as preclinical and clinical studies are recommended.

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Conflict of interest The author reports no conflicts of interest in this work.

References

1. Pleńkowska J, Gabig-Cimińska M, Mozolewski P. Oxidative Stress as an Important Contributor to the Pathogenesis of Psoriasis. *Int J Mol Sci*. 2020;21(17):6206.
2. Rendon A, Schäkel K. Psoriasis Pathogenesis and Treatment. *Int J Mol Sci*. 2019;20(6):1475.
3. Pual S, Sen S, Nath I, Kumar A, Biswas UK. Psoriasis, An inflammatory condition associated with oxidative stress. *Asian J Med Sci*. 2021;12(4):24-30.
4. Medovic MV, Jakovljevic VL, Zivkovic VI, et al. Psoriasis between Autoimmunity and Oxi-

dative Stress: Changes Induced by Different Therapeutic Approaches. *Oxid Med Cell Longev*. 2022;2022:2249834.
5. Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A. The Potential of Plant Phenolics in Prevention and Therapy of Skin Disorders. *Int J Mol Sci*. 2016;17(2):160.
6. Xian D, Guo M, Xu J, Yang Y, Zhao Y, Zhong J. Current evidence to support the therapeutic potential of flavonoids in oxidative stress-related dermatoses. *Redox Rep*. 2021;26(1):134-146.
7. Čižmárová B, Hubková B, Tomečková V, Birková A. Flavonoids as Promising Natural Compounds in the Prevention and Treatment of Selected Skin Diseases. *Int J Mol Sci*. 2023;24(7):6324.
8. Department of Thai Traditional and Alternative Medicine. Nationality Thai Traditional Medicine Formulary Special Edition to Commemorate the Auspicious Occasion of the Coronation of H.M. King Rama X, B.E.2562. Samcharoen Panich (Bangkok) Co., Ltd. 2020:97-98.
9. Cheng Y, Liu Y, Tan J, Sun Y, Guan W, Jiang P, et al. Integrated serum metabolomics and network pharmacology approach to reveal the potential mechanisms of withanolides from the leaves of Datura metel L. on psoriasis. *J Pharm Biomed Anal*. 2020;186:113277.
10. Saelee C, Thongrakard V, Tencomnao T. Effects of Thai medicinal herb extracts with anti-psoriatic activity on the expression of NF-κB signaling biomarkers in HaCaT keratinocytes. *Molecules*. 2011;16(5):3908-3932.
11. Mosaddik MA, Banbury L, Forster P, et al. Screening of some Australian Flacourtiaceae species for in vitro antioxidant, cytotoxic and antimicrobial activity. *Phytomedicine*. 2004;11(5):461-466.
12. Tamuly C, Hazarika M, Bora J, Bordoloi M, Boruah MP, Gajurel PR. In vitro study on antioxidant activity and phenolic content of three Piper species from North East India. *J Food Sci Technol*. 2015;52:117-128.
13. Tamuly C, Hazarika M, Bora J, Gajurel PR. Antioxidant Activities and Phenolic Content of *Piper wallichii* (Miq.) Hand.-Mazz. *Int J Food Prop*. 2014;17:309-320.
14. Klinthong S, Khammanit R, Phornchirasilp S, Temsiririrkkul R, Siriwatanametanon N. In vivo anti-inflammatory and in vitro antioxidant activities of a Thai traditional formula, Rid-si-duang-ma-ha-kan, for hemorrhoid treatment. *Mahidol Univ J Pharm Sci*. 2015;42(3):144-152.
15. Bhardwaj K, Kumar S, Ojha S. Antioxidant activity and FT-IR analysis of Datura innoxia and Datura metel leaf and seed methanolic extracts. *Afr J Tradit Complement Altern Med*. 2016;13(5):7-16.
16. Roland MN, Moussa C, Cristina M, Laurian V, Brânduşa T, Georges O. Antioxidant Capacity and Phenolic Composition of Crateva adansonii DC. (Capparaceae). *Int J Sci Res*. 2018;7(8): 493-497.
17. Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T. Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. *Chem Pharm Bull (Tokyo)*. 1994;42(8):1663-1665.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*. 1999;26(9):1231-1237.
19. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" : the FRAP assay. *Anal Biochem*. 1996;239(1):70-76.
20. Folin O, Ciocalteu V. On Tyrosine and tryptophane determinations in proteins. *J Biol Chem*. 1927;73:627-650.
21. Agbor A, Vinson A, Donnelly E. Folin-Ciocalteau reagent for polyphenolic assay. *Int J Food Sci Nutr Diet*. 2014;8:147-156.
22. Zhu H, Wang Y, Liu Y, Xia Y, Tang T. Analysis of flavonoids in Portulaca oleracea L. by UV-Vis spectrophotometry with comparative study on different extraction technologies. *Food Anal Methods*. 2010;3:90-97.
23. Umbert I, Márquez-Kisinousky L, Farrera C, Valls J, Valledor A, Fernández-Fernández L. New Treatment for psoriasis: therapeutic efficiency in patients and antiinflammatory effect in vitro. *Juniper Online J Dermatol Cosmet*. 2020;3(1):6-11.
24. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol*. 2011;48(4):412-422.

25. López-Alarcón C, Denicola A. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. *Anal Chim Acta*. 2013;763:1-10.
26. Bibi Sadeer N, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations. *Antioxidants (Basel)*. 2020;9(8):709.
27. Bié J, Sepodes B, Fernandes PCB, Ribeiro MHL. Polyphenols in Health and Disease: Gut Microbiota, Bioaccessibility, and Bioavailability. *Compounds*. 2023;3(1):40-72.
28. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects -A review. *J Funct Foods*. 2015;18:820-897.