EVALUATION OF THE IN VITRO ANTIOXIDANT CAPACITY OF INTHAJAWORN RECIPE A THAI TRADITIONAL CANNABIS MEDICINE AND PHYTOCHEMICAL ANALYSIS BY HPLC AND GCMS TECHNIQUES

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ABSTRACT

The medicinal plant is a source of bioactive compounds and represents an alternative used for the treatment of various diseases. In Thailand, there are several traditional medicine recipes such as Bhamrung-Lohit, Ya-Ha-Rak and Inthajaworn recipe. These recipes were used for treating many diseases such as antioxidant and anti-inflammatory activities, promoting strength and prolonging the lifespan. In this study, the Inthajaworn recipe was determined the chemical composition by HPLC and GCMS techniques and antioxidant capacity was analyzed by DPPH and ABTS assays. The correlation between chemical content and antioxidant capacity was analyzed. The result showed that the 6 phenolic and 2 flavonoid compounds were found in Inthajaworn recipe which is quercetin showed the highest content (239.88±0.43 µg/g extract). While the GCMS result was classified the chemical composition into 6 groups which are Alkaloids (55.71%), Miscellaneous (17.30%), Triterpenes (7.61%), Long chain hydrocarbons (3.14%), Cannabinoids (1.36%) and Sesquiterpenes (0.38%) group. Piperine was the highest abundant (48.08%). the Inthajaworn recipe presents the antioxidant capacity with DPPH and ABTS values of 26.91±0.24 and 55.25±1.82 mg TE/g extract, respectively. In addition, Pearson correlation analysis presented syringic acid, rutin and p-hydroxybenzoic acid were contributors to antioxidant capacity (DPPH and ABTS). Therefore, these result findings suggest that the Inthajaworn recipe is a source of natural antioxidant agents that could be used as an indicator of the antioxidant properties in the Inthajaworn recipe.

Keyword: Phytochemical, Antioxidant, Inthajaworn, HPLC, GCMS

INTRODUCTION

The primary source of tissue damage is oxidative stress, which is linked to several chronic illnesses, such as cancer, liver, neurological, diabetes mellitus (DM), and cardiovascular disorders. (Siddeeg et al., 2021). The cells, through metabolizing oxygen, create reactive species of oxygen (ROS), that are potentially harmful. High levels of ROS in biological cells have a large impact on their functioning, leading to deficient cell operation (Munteanu and Apetrei, 2021). Thus, the antioxidant agents are important for inhibiting, balancing or removing the ROS. The plant is one of the sources of antioxidant agents (Ayoub and Mehta, 2018).

Plants, vegetables, fruits, nuts, and spices are important sources of dietary active antioxidants that protect the body from oxidative stress-induced (Siddeeg et al., 2021). Since ancient times, medicinal plants and spices have been valued for both their flavor and aroma as well as their therapeutic properties. One of the benefits of medicinal plants is the antioxidant agents, also used for cooking and not only for medicine (Ulewicz-Magulska and Wesolowski, 2023). In addition, plant tissues consist of different antioxidant agents such as phenolic, flavonoids, tannins and lignin precursors, which act as ROS-scavenging compounds (Basu and Maier, 2016; Tajner-Czopek et al., 2020).

Animistic beliefs and old holistic practices have influenced the growth of Thai traditional medicine. In China and India, traditional medicine has been successfully integrated into the healthcare system. Some Thai traditional medicine was used to renew the body, enhance strength, avoid ailments, and extend the lifetime or longevity (Ruangchuay et a., 2021). A longevity remedy in Thai traditional medicine has been associated with body health nourishment, appetizing, muscle pain relief, relaxation, normal excretion, body health improvement, and, most importantly, long-life aging. In addition, this remedy has been described as possessing anti-aging properties (Konsue and Taepongsorat, 2022). Moreover, National Thai traditional remedies with cannabis have several recipes for longevity which are Mahawattana, Kaedhatuphikar and Inthajaworn recipes (Sripan et al., 2022). The Thai traditional medicine for longevity had a few reported biological activities.

OBJECTIVES

2.1 To determine the antioxidant capacity of the Inthajaworn recipe by DPPH and ABTS assays

2.2 To analyze the chemical composition of the Inthajaworn recipe by HPLC and GCMS techniques

MATERIAL AND METHODS

3.1 Inthajaworn recipe preparation

The composition of Inthajaworn recipe was described in National Thai traditional remedies with cannabis in 2021. The medicinal plants were purchased from Healthy Hills Farm Company Limited, Bangkok, Thailand. The cannabis leaves were provided from Taratera Corporation Company Limited, Bangkok, Thailand.

The Inthajaworn recipe consists of 8 medicinal plants which are *Ferula assafoetida* gum 1.00 g, *Acorus calamus* rhizomes 2.00 g, *Ardisia elliptica* fruit 2.00 g, *Zingiber ligulatum* rhizomes 2.00 g, *Piper nigrum* fruit 1.00 g, *Cannabis sativa* leaves 1.00 g, *Piper longum* fruit 4.46 g and *Amorphophallus paeoniifolius* tuber 37.00 g (Sripan et al., 2022).

3.2 Extraction

Briefly, the Inthajaworn powder 50 g was macerated with 95% ethanol 100 mL by sonication technique for 30 min (3 times) which was controlled at an ultrasonic frequency of 40 kHz and 220 Volts by Ultrasonic cleaners (GT sonic®, Guangdong, China). Then, extract was filtered with Whatman No. 1 filter paper and evaporate to dry the filtrate by using rotary evaporator to obtain Inthajaworn ethanolic extract.

3.3 Chemical analysis

3.3.1 HPLC analysis

Phenolic acid and flavonoid contents were conducted on a HPLC (Shimadzu Prominence-i LC-2030C 3D; Kyoto, Japan) with column Unisol C18, 5 μm particle size, 250×4.6 mm (Torrance, Ca, USA). The purified water with 1% acetic acid (v/v) (solvent A) and acetonitrile (solvent B) were used for the mobile phase. The HPLC gradient program was from 0 to 5 min, 5% solvent B; from 5-15, 9% solvent B; from 15-22, 11% solvent B; from 22-38, 18% solvent B; from 38-43, 23% solvent B; from 43-44, 90% solvent B; from 44-45, 80% solvent B; from 45-55, isocratic program at 80% solvent B; from 55-65, re-equilibration at 5% solvent B; linear gradient from 65-70, 5% solvent B. The sample was injected in a volume of 20 μL into a column at a temperature 40°C with a flow rate of 0.8 mL/min. The respective UV-diode array detection wavelengths were 280 nm for hydroxybenzoic acids, 320 nm for hydroxycinnamic acids and 370 nm for flavonoids. The HPLC chromatogram between extracts and standard compounds were compared. The presence of phenolic compounds in the extract was determined based on the retention time (Siriparu et al., 2022).

3.3.2 GCMS analysis

GCMS analysis of extract was performed by using a SHIMADZU QP-2010 instrument (Kyoto, Japan). The sample was injected in a volume of 1 μL into Agilent J&W DB-5MS column (30 m×0.25 mm, 0.25 μm, Santa Clara, CA, USA) with a flow rate was 1 mL/min and helium 5.5 was a carrier gas. The split ratio was set to 1:20. The oven temperature program was set as follows: starting temperature 70°C (hold time 2 min), with gradual increases in temperature from 70 to 200°C (5.0°C /min, hold 10 min), from 200 to 230°C (5.0°C/min, hold 10 min), from 230 to 250°C (5.0°C/min, hold 5 min), from 250 to 320°C (5.0°C/min, hold 20 min). The peaks were analyzed based on GC retention time and mass spectral identity was by comparison to the NIST17.LIB library (Palmieri, et al., 2021).

3.4 Antioxidant capacities

3.4.1 DPPH radical scavenging

The mixture of extract and 0.2 mM DPPH were added into 96 well plates and incubated for 30 min in dark conditions. Then, the mixture was read with a microplate reader (EZ Read 2000, Biochrom, USA) at 517 nm. The antioxidant capacity was calculated in mg TE/g extract unit (Puthongking et al., 2023).

3.4.2 ABTS radical scavenging

The radical cation ($ABTS^{\cdot+}$) was prepared from the mixture of ABTS and potassium persulfate in purified water for 12-16 hours before use. The mixture of extract and $ABTS^{\cdot+}$ were added into 96 well plates and incubated for 10 min in dark conditions. Then, the mixture was read with a microplate reader at 735 nm. The antioxidant capacity was calculated in mg TE/g extract unit (Yongram et al., 2022).

3.5 Statistical analysis

The results were presented as mean ± SD. The statistical analysis was done by one-way ANOVA with Tukey HSD and t-Test to compare the differences between sample groups. The differences were considered to be significant at $p<0.05$. The correlation analysis results of antioxidant capacity with phenolic and flavonoid contents were expressed as Pearson correlation coefficients using SPSS 23.0 software for Windows. (SPSS Inc., Chicago, IL, USA).

RESULTS

4.1 HPLC analysis

The Inthajaworn extract was evaluated for the phenolic and flavonoid content by HPLC technique which are 9 phenolic group (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, chlorogenic acid, sinapinic acid, p-coumaric acid and ferulic acid) and 2 flavonoid group (rutin and quercetin). The result showed that the Inthajaworn extract had a range of chemical content of 91.77 to 239.88 µg/g extract. The quercetin (239.88±0.43 µg/g extract) showed the significantly highest content followed by gallic acid (138.00±6.92 µg/g extract), p-hydroxybenzoic acid (134.43±0.93 µg/g extract), syringic acid (128.78±0.48 µg/g extract), protocatechuic acid (113.68±0.05 µg/g extract), ferulic acid (102.15±0.80 µg/g extract), p-coumaric acid (95.84±0.28 µg/g extract), rutin (91.77±0.11 µg/g extract), respectively. However, vanillic acid, chlorogenic acid and sinapinic acid are not found in the Inthajaworn recipe (Figure 1). In addition, the total phenolic and flavonoid compounds showed of relative abundance of 1044.52±6.64 µg/g extract. The structure of chemical composition in Inthajaworn extract by HPLC analysis showed in Figure 2.

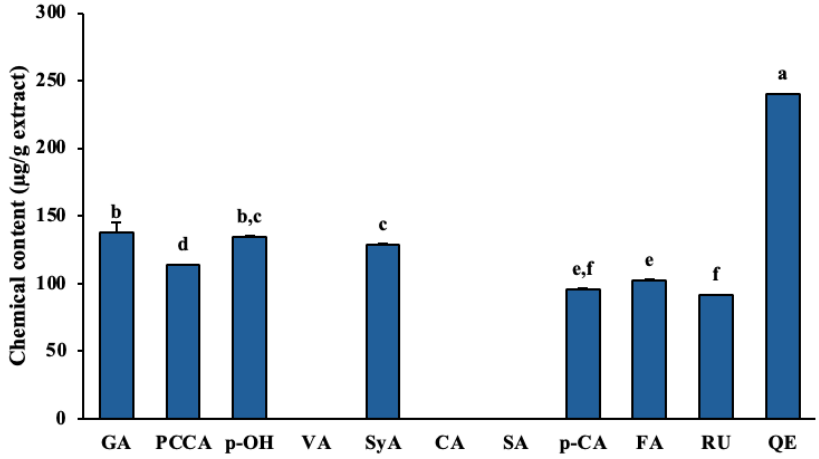

Figure 1. HPLC analysis of phenolic and flavonoid compound of Inthajaworn recipe. Gallic acid (GA), protocatechuic acid (PCCA), p-hydroxybenzoic acid (p-HO), vanillic acid (VA), syringic acid (SyA), chlorogenic acid (CA), sinapinic acid (SA), p-coumaric acid (p-CA) and

ferulic acid (FA) and flavonoid such as rutin (RU) and quercetin (QE). Different letters indicate a significant difference between each content ($p<0.05$).

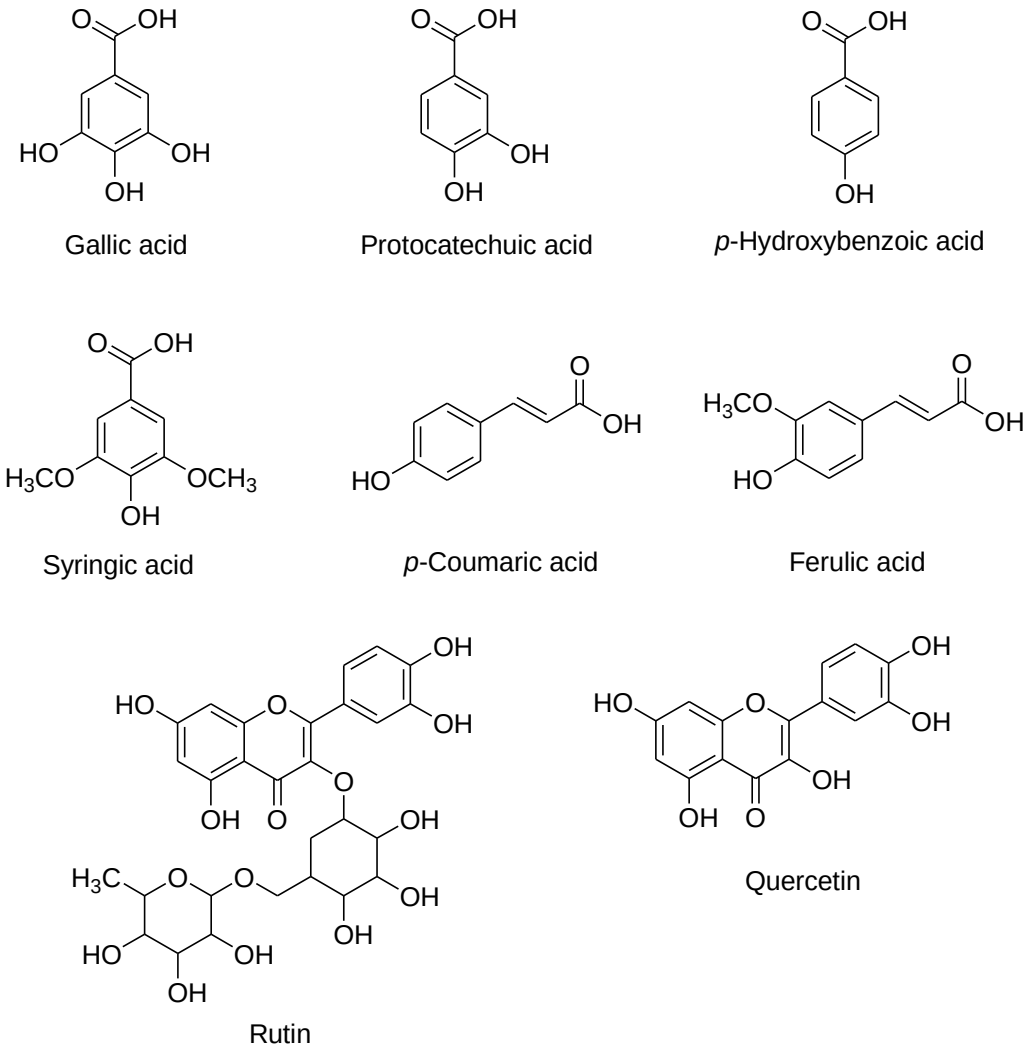

Figure 2. The chemical structure of phenolic and flavonoid compounds of Inthajaworn recipe by HPLC analysis

4.2 GCMS analysis

The 30 compounds were identified by GCMS analysis (Table 1). The 8 most abundant compounds were piperine (48.08%), asarone (14.57%), stigmasterol (4.30%), (2E,4E,10E)-N-isobutylhexadeca-2,4,10-trienamide (3.33%), (14R)-14-methyl hexadec-8-yn-1-ol (2.88%), glyceraldehyde (2.11%), γ-sitosterol (1.84%) and (2E,4E,12E)-1-(piperidin-1-yl)octadeca-2,4,12-trien-1-one (1.72%) in Figure 3. Moreover, the GCMS result was classified the chemical composition into 6 groups such as alkaloids (55.71%), triterpenes (7.61%), sesquiterpenes (0.38%), cannabinoids (1.36%), long chain hydrocarbons (3.14%) and miscellaneous (17.30%) group in Figure 4. The major abundant in each group which are piperine (48.08%), stigmasterol (4.30%), 2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol (0.13%), cannabinol (0.87%), (R)-(-)-14-Methyl-8-hexadecyn-1-ol (2.88%) and asarone (14.57%), respectively.

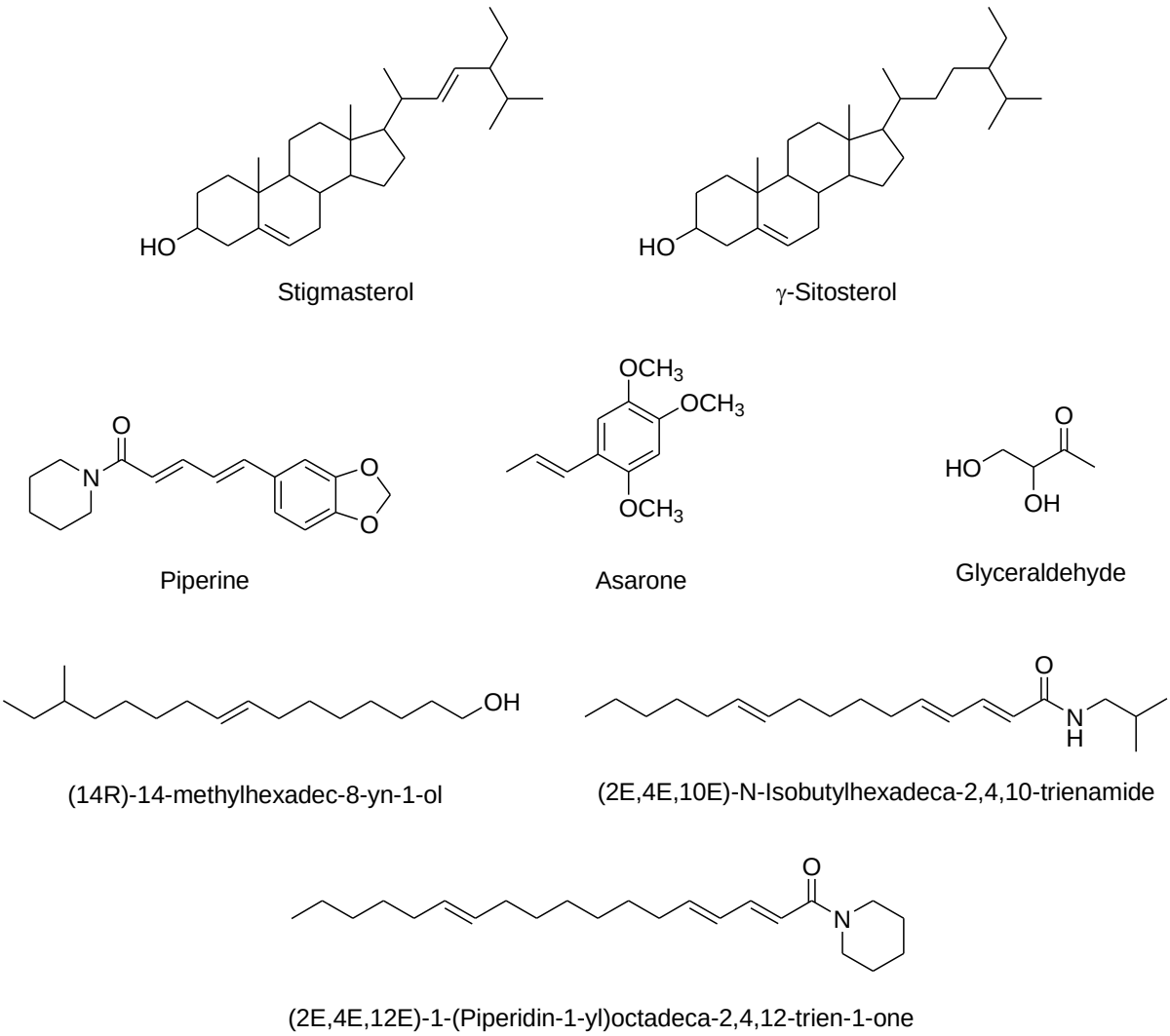

Figure 3. The chemical structure of 8 most abundant compounds in Inthajaworn recipe by GCMS analysis

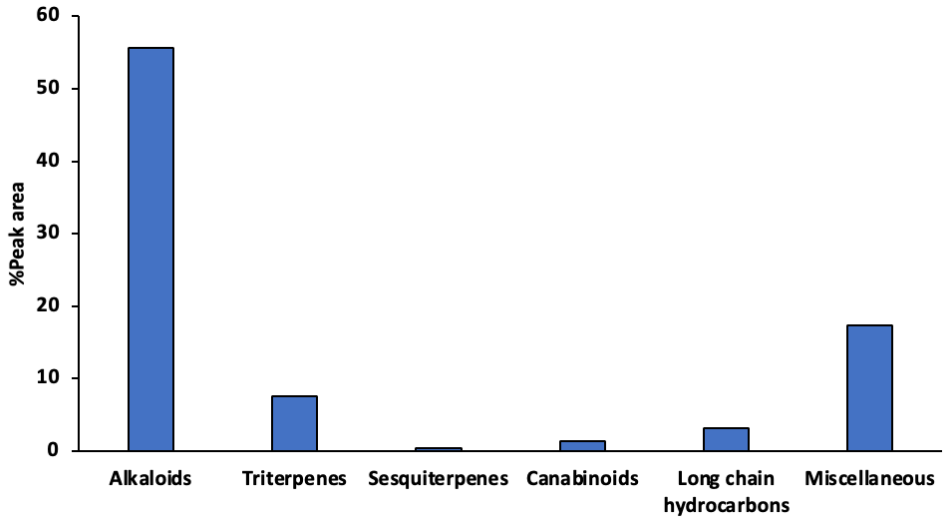

Figure 4. The chemical classification group in Inthajaworn recipe by GCMS analysis.

Table 1. Chemical composition in Inthajaworn recipe GCMS analysis

No.	Rt (min)	CAS Registry	Formula	MW	KI	%Peak area	Compound name
Sesquiterpenes							
1	21.16	1139-30-6	$C_{15}H_{24}O$	220	1507	0.12	Caryophylleneoxide
2	22.22	88395-46-4	$C_{15}H_{24}O$	220	1569	0.05	Isospathulenol
3	22.45	19431-79-9	$C_{15}H_{24}O$	220	1677	0.04	Caryophylla-4(12),8(13)-dien-5.alpha.-ol
4	22.85	-	$C_{15}H_{26}O$	222	1598	0.13	2-(4a,8-Dimethyl-2,3,4,5,6,8a-hexahydro-1H-naphthalen-2-yl)propan-2-ol
5	24.92	25330-21-6	$C_{15}H_{26}O_2$	238	1725	0.04	Isocalamenediol
Total sesquiterpene						0.38%	
Long chain hydrocarbons							
6	30.10	628-97-7	$C_{18}H_{36}O_2$	284	1978	0.07	Hexadecanoic acid,ethyl ester
7	35.53	544-35-4	$C_{20}H_{36}O_2$	308	2193	0.19	Linoleic acid ethyl ester
8	58.76	64566-18-3	$C_{17}H_{32}O$	252	1907	2.88	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
Total long chain hydrocarbon						3.14%	
Cannabinoids							
9	45.48	20675-51-8	$C_{21}H_{30}O_2$	314	2486	0.25	Cannabichromene (CBC)
10	48.32	1972-08-3	$C_{21}H_{30}O_2$	314	2475	0.24	Tetrahydrocannabinol (THC)
11	50.87	521-35-7	$C_{21}H_{26}O_2$	310	2582	0.87	Cannabinol (CBN)
Total cannabinoids						1.36%	
Alkaloids							
12	27.81	137-58-6	$C_{14}H_{22}N_2O$	234	1966	0.65	Lidocaine
13	52.53	23512-46-1	$C_{17}H_{21}NO_3$	278	2391	0.67	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one
14	58.21	943546-13-2	$C_{20}H_{35}NO$	305	2369	3.33	(2E,4E,10E)-N-Isobutylhexadeca-2,4,10-trienamide
15	59.67	52657-12-2	$C_{19}H_{33}NO$	291	2277	0.20	(2E,4E)-1-(Piperidin-1-yl)tetradeca-2,4-dien-1-one
16	63.38	94-62-2	$C_{17}H19NO_3$	285	2399	48.08	Piperine
17	66.03	943546-21-2	$C_{24}H_{43}NO$	361	2767	0.84	(2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide
18	66.56	943546-19-8	$C_{23}H_{39}NO$	345	2682	1.72	(2E,4E,12E)-1-(Piperidin-1-yl)octadeca-2,4,12-trien-1-one
19	67.27	151391-73-0	$C_{23}H_{41}NO$	347	2674	0.22	(2E,4E)-1-(Piperidin-1-yl)octadeca-2,4-dien-1-one
Total alkaloids						55.71%	
Triterpenes							
20	68.50	7144-08-3	$C_{28}H_{45}ClO_2$	448	2813	0.06	Cholest-5-en-3-ol(3β)-,carbonochloridate
21	71.45	474-62-4	$C_{28}H_{48}O$	400	2632	0.86	Campesterol

No.	Rt (min)	CAS Registry	Formula	MW	KI	%Peak area	Compound name
22	71.97	83-48-7	$C_{29}H_{48}O$	412	2739	4.30	Stigmasterol
23	73.08	83-47-6	$C_{29}H_{50}O$	414	2731	1.84	γ-Sitosterol
24	75.17	1058-61-3	$C_{29}H_{48}O$	412	2714	0.35	Stigmast-4-en-3-one
25	75.52	13159-28-9	$C_{30}H_{48}O_2$	440	3036	0.20	Betulinaldehyde
Total triterpenes						7.61%	
Miscellaneous							
26	3.07	56-82-6	$C_3H_6O_3$	90	913	2.11	Glyceraldehyde
27	20.69	5353-15-1	$C_{12}H_{16}O_3$	208	1550	0.07	γ-Asarone
28	21.76	2883-98-9	$C_{12}H_{16}O_3$	208	1568	14.57	Asarone
29	41.21	555-66-8	$C_{17}H_{24}O_3$	276	2242	0.30	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one
30	69.81	59-02-9	$C_{29}H_{50}O_2$	430	3149	0.25	VitaminE
Total miscellaneous						17.31%	

Note: Rt as retention time, MW as molecular weight and KI as Kovats index.

4.3 Antioxidant capacity

The antioxidant capacity of Inthajaworn recipe was determined by DPPH and ABTS radical scavenging assays. The result showed that the Inthajaworn recipe has a DPPH value of 26.91±0.24 mg TE/g extract. DPPH value was calculated for a standard curve of Trolox as $y = -0.0478x + 0.5172$, $R^2 = 0.9998$. For ABTS value, the result demonstrated the high antioxidant capacity with a value of 55.25±1.82 mg TE/g extract, which is significantly higher than DPPH value (Figure 5). Also, ABTS value was calculated for a standard curve of Trolox as $y = -0.0764x + 0.5528$, $R^2 = 0.9958$.

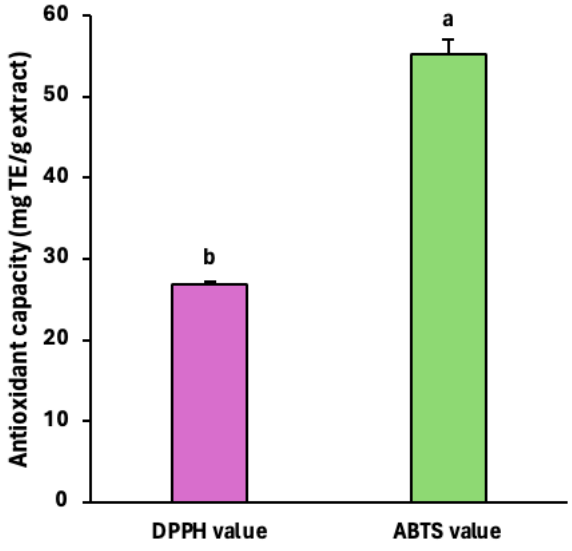

Figure 5. Antioxidant capacity of Inthajaworn recipe. Different letters indicate a significant difference between DPPH (■) and ABTS (■) value ($p<0.05$).

4.4 Correlation analysis

The correlation coefficient (r) between the antioxidant capacities revealed by the two assays (ABTS and DPPH) and phenolic and flavonoid are represented in Table 2. The correlation criteria of 3 correlation levels were defined as strong correlation (r = (+/–) 0.600-1.000), moderate correlation (r = (+/–) 0.400-0.599), and weak correlation (r = (+/–) 0.000-0.399) (Ngamdee et al., 2016). Syringic acid showed a positive strong correlation between antioxidant capacities (r = 0.994 for DPPH and r = 0.955 for ABTS), protocatechuic acid and ABTS (r = 0.922), p-hydroxybenzoic acid and DPPH (r = 0.925), rutin with DPPH (r = 0.978) and ABTS (r = 0.810). Also, protocatechuic acid with DPPH (r = 0.688) and p-hydroxybenzoic acid with ABTS (r = 0.692), Moreover, it found a moderate correlation of ferulic acid with ABTS (r = 0.461) including the low correlation was ferulic acid with DPPH (r = 0.064). However, the quercetin showed a negative strong correlation with DPPH (r = -1.000), significantly ($p<0.01$). Inaddition, gallic acid, p-coumaric acid and demonstrated the negative correlation with ABTS and DPPH in Table 2.

Table 2. Correlation analysis of chemical content and antioxidant capacity

Compounds	Pearson correlation coefficient (r)	
	Antioxidant capacities	
	DPPH	ABTS
Gallic acid (GA)	-0.828	-0.532
Protocatechuic acid (PCCA)	0.688	0.922
p-Hydroxybenzoic acid (p-HO)	0.925	0.692
Syringic acid (SyA)	0.994	0.955
p-Coumaric acid (p-CA)	-0.928	-0.700
Ferulic acid (FA)	0.064	0.461
Rutin (RU)	0.978	0.810
Quercetin (QE)	-1.000**	-0.912

Note: ** is significant at the $p<0.01$ (2-tailed)

DISCUSSION

Phenolic acid (such as gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, chlorogenic acid, sinapinic acid, p-coumaric acid and ferulic acid) and flavonoid (such as rutin and quercetin) were determined by HPLC. Benzoic and cinnamic acid derivatives are phenolic compounds endogenous to cereal grains, oilseeds, pulses/legumes, vegetables and other plant species. The phenolic acids exhibited radical scavenging activity which is gallic acid showing the highest activity followed by gentisic acid, syringic acid, caffeic acid, protocatechuic acid, sinapic acid, ferulic acid, isoferulic acid, vanillic acid, p-coumaric acid, o-coumaric acid, m-coumaric acid and p-hydroxybenzoic acid, respectively by DPPH assay. (Karamać et al., 2005). Moreover, their phenolic and flavonoid were found in *Dipterocarpus alatus* leaves, bark and twig (Puthongking et al., 2023), *Zea mays* L. and *Clitoria ternatea* (Ratha et al., 2023), mung bean sprouts (Siriparu et al., 2022) and *Morus alba* L. fruit (Yongram et al., 2022). In addition, Inthajawor recipe was reported the cannabinoid content in

range of 0.48 to 2.05 mg/g extract which is CBDV showed the highest cannabinoid content (Sripan et al., 2022).

GCMS analysis, the result classified the chemical composition into 6 groups which are alkaloids which is highly abundant followed by miscellaneous, triterpenes, long chain hydrocarbons, cannabinoids and sesquiterpenes group. Alkaloids group can behave both as antioxidants and pro-oxidants properties (Macáková et al., 2019). Terpenes and triterpenes group reported antioxidant and anti-inflammatory activities (González-Burgos and Gómez-Serranillos, 2012; Park et al., 2023). Sesquiterpenes group showed potential antioxidant activity (Khan et a., 2008). Also, the cannabinoids group were antioxidant agents and had intervention abilities in antioxidant action (Dawidowicz et al., 2021). In addition, the compounds have a peak area than 1% which are piperine, asarone, stigmasterol, (2E,4E,10E)-N-isobutyl hexadeca-2,4,10-trienamide, (14R)-14-methylhexadec-8-yn-1-ol, glyceraldehyde, γ-sitosterol and (2E,4E,12E)-1-(piperidin-1-yl)octadeca-2,4,12-trien-1-one. These compounds have been reported the biological activities such as piperine showed an anti-inflammatory effect by inhibiting or quenching free radicals, ROS and hydroxyl radicals (Mittal and Gupta, 2000). Asarone exhibit multiple pharmacological properties including antioxidant, anti-inflammatory, antiapoptotic, anticancer, and neuroprotective effects (Balakrishnan et al., 2022). Stigmasterol was indicated potent pharmacological effects such as anticancer, anti- inflammatory, anti- diabetic, immunomodulatory, antiparasitic, antifungal, antibacterial and antioxidant (Bakrim et al., 2022). γ-Sitosterol displays a potential anticancer activity via cell cycle arrest and apoptosis cell death (Sundarraj et al., 2012).

For antioxidant capacity, the Inthajawor recipe showed the high antioxidant capacity in ABTS assay than DPPH assay. Moreover, Inthajawor recipe was reported the antioxidant activity via DPPH and ABTS assays with IC_{50} value of 188.71±2.45 and 54.21±1.81 µg/mL, respectively, including FRAP value of 107.95±5.94 mmol/100 g extract (Sripan et al., 2022). The formula for the longevity of Thai traditional medicine showed modulated antioxidant activity with IC_{50} value of 187.62 µg/mL by DPPH assay (Luanchoy et al., 2014) which is similar to the antioxidant activity of Inthajawor recipe.

In correlation analysis, the presence of low and moderate correlations of some phenolic and flavonoids have been reported *in vitro* antioxidant activities in plant extracts. For these reasons, the antioxidant activity of Inthajawor recipe was considered to be caused by the combined effects of phenolic, flavonoid and other compounds (Ngamdee et al., 2016). Our result showed positive and negative correlation. The negative correlation means the higher value of phenolic acid and flavonoid contents might give a lower antioxidant capacity value (Indradi et al., 2017).

CONCLUSION

Inthajawor recipe demonstrated a good antioxidant capacity by DPPH and ABTS assays. The correlations between antioxidant capacity determined by DPPH and ABTS with the phenolic acid and flavonoid compounds confirm that phenolic compounds contribute to the antioxidant properties of the Inthajawor recipe. Moreover, the Inthajawor recipe presents several chemical compositions that are used information for the development of pharmaceutical products.

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REFERENCES

Ayoub, Z., Mehta, A. (2018). Medicinal plants as potential source of antioxidant agents: a review. Asian journal of pharmaceutical and clinical research, 11(6), 50-56.

Bakrim, S., Benkhaira, N., Bourais, I., Benali, T., Lee, L.H., El Omari, N., Sheikh, R.A., Goh, K.W., Ming, L.C., Bouyahya, A. (2022). Health benefits and pharmacological properties of stigmasterol. Antioxidants, 11(10), 1912.

Balakrishnan, R., Cho, D.Y., Kim, I.S., Seol, S.H., Choi, D.K. (2022). Molecular mechanisms and therapeutic potential of α- and β-asarone in the treatment of neurological disorders. Antioxidants, 11, 281.

Basu P, Maier C. (2016). *In vitro* antioxidant activities and polyphenol contents of seven commercially available fruits. Pharmacognosy research, 8(4), 258-264.

Dawidowicz, A.L., Olszowy-Tomczyk, M., Typek, R. (2021). CBG, CBD, Δ9-THC, CBN, CBGA, CBDA and Δ9-THCA as antioxidant agents and their intervention abilities in antioxidant action. Fitoterapia. Jul;152, 104915.

González-Burgos, E., Gómez-Serranillos, M.P. (2012). Terpene compounds in nature: a review of their potential antioxidant activity. Current medicinal chemistry, 19(31), 5319-5341.

Indradi, R.B., Fidrianny, I., Wirasutisna, K.R. (2017). DPPH scavenging activities and phytochemical content of four Asteraceae plants. International journal of pharmacognosy and phytochemical research, 9(6), 755-759.

Karamać, M., Kosinska, A., Pegg, R.B. (2005). Comparison of radical-scavenging activities for selected phenolic acids. Polish journal of food and nutrition sciences, 14/55(2), 165-170.

Khan, H., Khan, A.L., Hussain, J., Adnan, M., Hussain, I., Khan, T., Khan, A.R. (2008). Review Sesquiterpenes: The potent antioxidants. Pakistan journal of scientific and industrial research, 51(6), 343-350.

Konsue, A., Taepongsorat, L. (2022). Phytochemical screening and antioxidant activity of longevity remedy from national Thai traditional medicine scripture (formulary special edition). Tropical journal of natural product research, 6(6), 868-871.

Luanchoy, S., Tiangkul, S., Wongkrajang, Y., Temsiririrkkul, R., Peungvicha, P., Nakornchai, S. (2014). Antioxidant activity of a Thai traditional formula for longevity. Mahidol University journal of pharmaceutical sciences, 41(3), 1-5.

Macáková, K., Afonso, R., Saso, L., Mladěnka, P. (2019). The influence of alkaloids on oxidative stress and related signaling pathways. Free radical biology and medicine, 134, 429-444.

Mittal, R., Gupta, R.L. (2000). *In vitro* antioxidant activity of piperine. Methods and findings in experimental and clinical pharmacology, 22(5), 271-274.

Munteanu, I.G., Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: a review. International journal of molecular sciences, 22(7), 3380.

Ngamdee, P., Wichai, U., Jiamyangyuen, S. (2016). Correlation between phytochemical and mineral contents and antioxidant activity of black glutinous rice bran, and its potential chemopreventive property. Food technology and biotechnology, 54(3), 282-289.

Palmieri, S., Maggio, F., Pellegrini, M., Ricci, A., Serio, A., Paparella, A., Sterzo, C.L. (2021). Effect of the distillation time on the chemical composition, antioxidant potential and antimicrobial activity of essential oils from different *Cannabis sativa* L. cultivars. Molecules, 26(16), 4770.

Park, J.S., Rehman, I.U., Choe, K., Ahmad, R., Lee, H.J., Kim, M.O. (2023). A triterpenoid lupeol as an antioxidant and anti-neuroinflammatory agent: impacts on oxidative stress in Alzheimer's disease. Nutrients, 15(13), 3059.

Puthongking, P., Ratha, J., Panyatip, P., Datham, S., Siriparu, P., Yongram, C. (2023). The effect of extraction solvent on the phytochemical contents and antioxidant and acetylcholinesterase inhibitory activities of extracts from the leaves, bark and twig of *Dipterocarpus alatus*. Tropical journal of natural product research, 7(12), 5595-5600.

Ratha, J., Yongram, C., Panyatip, P., Powijitkul, P., Siriparu, P., Datham, S., Priprem, A., Srisongkram, T., Puthongking, P. (2023). Polyphenol and tryptophan contents of purple corn (*Zea mays* L.) variety KND and butterfly pea (*Clitoria ternatea*) aqueous extracts: insights into phytochemical profiles with antioxidant activities and PCA analysis. Plants, 12(3), 603.

Ruangchuay, S., Wang, Q.Q., Wang, L.Y., Lin, J., Wang, Y.C., Zhong, G.H., Maneenoon, K., Huang, Z.B., Chusri, S. (2021). Antioxidant and antiaging effect of traditional Thai rejuvenation medicines in *Caenorhabditis elegans*. Journal of integrative medicine, 19(4), 362-373.

Siddeeg, A., AlKehayez, N.M., Abu-Hiamed, H.A., Al-Sanea, E.A., Al-Farga, A.M. (2021). Mode of action and determination of antioxidant activity in the dietary sources: an overview. Saudi journal of biological sciences, 28(3), 1633-1644.

Siriparu, P., Panyatip, P., Pota, T., Ratha, J., Yongram, C., Srisongkram, T., Sungthong, B., Puthongking, P. (2022). Effect of germination and illumination on melatonin and its metabolites, phenolic content, and antioxidant activity in mung bean sprouts. Plants, 11, 2990.

Sripan, P., Yongram, C., Chokchaisiri, S., Meeboonya, R., Wonganan, O., Luangpirom, N., Kamoltham, T., Roongpisuthipong, A., Panyatip, P., Puthongking, P. (2022). Cannabinoids analysis, pharmacokinetic prediction and antioxidant activity of elixir Thai traditional cannabis recipes. Journal of Allied Health Sciences Suan Sunandha Rajabhat University, 8(2), 18-34.

Sundarraj, S., Thangam, R., Sreevani, V., Kaveri, K., Gunasekaran, P., Achiraman, S., Kannan, S. (2012). γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. Journal of ethnopharmacology, 141(3), 803-809.

Tajner-Czopek, A., Gertchen, M., Rytel, E., Kita, A., Kucharska, A.Z., Sokół-Łętowska, A. (2020). Study of antioxidant activity of some medicinal plants having high content of caffeic acid derivatives. Antioxidants, 9(5), 412.

Ulewicz-Magulska, B., Wesolowski, M. (2023). Antioxidant activity of medicinal herbs and spices from plants of the Lamiaceae, Apiaceae and Asteraceae families: chemometric interpretation of the data. Antioxidants, 12, 2039.

Yongram, C., Panyatip, P., Siriparu, P., Ratha, J., Sungthong, B., Tadtong, S., Puthongking, P. (2022). Influence of maturity stage on tryptophan, phenolic, flavonoid, and anthocyanin content, and antioxidant activity of *Morus alba* L. fruit. Rasayan journal of chemistry, 15(3), 1693-1701.