Antioxidant and Chemical contents of Thai Traditional Cannabis Recipes: Sanan Tripop oil

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Abstract

The present study investigated the chemical composition and antioxidant activity of the Sanan Tripop oil recipe, a traditional Thai medicinal preparation historically used for the treatment of Kasai Lek, a condition characterized by abdominal pain, rigidity, and digestive dysfunction. The oil was prepared from 17 medicinal plants, including *Cannabis sativa*, *Ocimum* spp., and *Boesenbergia* rotunda, extracted in sesame oil. Antioxidant activity was assessed using DPPH and ABTS radical scavenging assays and ferric reducing ability power (FRAP) assay, while phytochemical constituents, including total phenolics, flavonoids, chlorophylls, and carotenoids, were quantitatively analyzed. The results demonstrated weak antioxidant activity, with no observable effect in the DPPH assay (IC_{50} >1000 µg/mL) and low activity in the ABTS assay (IC_{50} = 825.04±24.65 µg/mL), also, FRAP value is 13.24±0.78 mmol Fe^{2+}/100 g extract. Phytochemical analysis revealed total phenolics of 29.38±1.15 mg GAE/g extract, total flavonoids of 90.40±0.54 mg QE/g extract, total chlorophylls of 0.08±0.00 mg/g extract, and total carotenoids of 0.03±0.00 mg/g extract. In conclusion, although the Sanan Tripop oil recipe exhibits limited free radical scavenging activity, its phytochemical profile indicates potential for other bioactive effects, supporting the need for further pharmacological and *in vivo* investigations to clarify its therapeutic applications in integrative medicine.

Keywords: Sanan Tripop oil, Antioxidant, Chemical contents, Thai Traditional medicine

1. Introduction

According to Thai medical theory, cannabis has been used for centuries in Thai traditional medicine, mostly as a component of multi-herb formulations that are intended to reduce pain, increase appetite, enhance sleep, and balance the body's elements. To lessen toxicity and increase therapeutic benefits, cannabis was rarely used as a single herb in traditional practice. Instead, it was mixed with other medicinal plants in carefully balanced recipes (Duangdamrong et al.,2022).

After cannabis was legalized for medical use again in 2019, the Ministry of Public Health has recognized and approved a number of official Thai traditional cannabis recipes. Ya Kae Lom Kae Sen, Suk Sai Yad, and Sanan TriPop are some of the recipes that are meant to help with certain health problems. For instance, some are given to help with pain, muscle tension, appetite, or as a supportive treatment for insomnia and stomach problems (Department of Thai Traditional and Alternative Medicine, 2019).

Pharmacological research indicates that cannabinoids (including THC and CBD) function synergistically with other herbal components in these formulations, thereby endorsing their application in contemporary integrative medicine (Russo et al., 2011). Nonetheless, clinical evidence is still scarce, necessitating additional research to confirm safety, efficacy, and suitable dosage recommendations. the integration of Thai traditional cannabis recipes into the healthcare system highlights both the cultural heritage of Thai medicine and the global trend of revisiting traditional remedies through modern scientific evaluation. This approach aims to preserve traditional wisdom while creating evidence-based pathways for safe and effective therapeutic use.

The Sanan Tripop oil recipe was used for treatment of Kasai Lek, a condition caused by compressed and hardened wind accumulating in the lower abdomen. The patient experiences pain, abdominal rigidity extending up to the chest, and inability to eat (Paratang et al.,2022). Therefore, this research was determined the antioxidant activity using DPPH and ABTS assays. The Total phenolic, flavonoid, chlorophyll and carotenoid contents were analyzed.

1.2 Research Objective

The objectives of this study were to analyze the antioxidant properties and chemical composition of Sanan Tripop oil recipe.

2. Materials and methods

2.1 Sample preparation

Sanan Tripop oil recipe was provided from Dejasit Thai traditional medicine clinic, Chiang Mai, Thailand during September 2025. The Sanan Tripop oil recipe was consist of *Ocimum tenuiflorum* leaves 1 kg, *Ocimum basilicum* leaves 1 kg, *Cleome viscosa* leaves 1 kg, *Boesenbergia rotunda* rhizome 1 kg, *Cannabis sativa* inflorescence 1 kg, *Piper nigrum* fruit 1 kg, *Allium ascalonicum* bulb 1 kg, *Leersia hexandra* leaves 1 kg, *Oxyceros horridus* fruit 1 kg, Salt 1 kg, *Myristica fragrans* seed 3.75 g, *Amomum krervanh* fruit 3.75 g, *Syzygium aromaticum* clove 3.75 g, *Cuminum cyminum* fruit 3.75 g, *Nigella sativa* seed 3.75 g, Camphor 3.75 g and Sesame oil 1 L.

2.2 Antioxidant activities

2.2.1 DPPH scavenging radical assay

The 200 mM DPPH was mixed with the sample (5-1000 µg/ml) in 96 well plate. The solution was incubated for 30 min in dark conditions. After 30 min, the solution was read at 590 nm using a microplate reader. The Trolox was a positive control and IC_{50} was calculated and DPPH value was calculated from Trolox standard curve. (Puthongking et al., 2023).

2.2.2 ABTS scavenging radical assay

The 7 mM ABTS radical cation was generated by mixed with potassium persulfate in purified water in dark conditions for 16-18 h. The ABTS radical cation mixed with the sample (5-1000 µg/ml) in 96 well plate. The solution was incubated for 10 min in dark conditions. After 10 min, the solution was read at 743 nm using a microplate reader. The Trolox was a positive control and IC_{50} was calculated and ABTS value was calculated from Trolox standard curve (Yongram et al., 2025).

2.2.3 Ferric Reducing Ability Power (FRAP) assay

The FRAP reagent was freshly prepared before each measurement by mixing acetate buffer (300 mM), TPTZ (10 mM in HCl (40 mM)), and $FeCl_3$ (20 mM in dist. water) in a ratio of 10:1:1 (v/v/v). For the analysis, the sample was mixed with FRAP reagent in 96 well plate and incubated for 5 min in dark conditions. The solution was read at 595 nm using a microplate reader. The Trolox was a positive control. The FRAP value was shown as mmol Fe^{2+}/100 g extract unit (Rumpf et al.,2023)

2.3 Chemical contents

2.3.1 Total phenolic content

The sample was mixed with 10%Folin-Ciocalteu reagent in 96 well plate and 7% sodium carbonate and incubated for 30 min in dark conditions. After 30 min, the solution was read at 760 nm using a microplate reader. The total phenolic content is shown as gallic acid equivalents (mg GAE/g extract) (Sripan et al., 2025).

2.3.2 Total flavonoid content

The sample was mixed with 2%Aluminium chloride in 96 well plate and incubated for 20 min in dark conditions. After 20 min, the solution was read at 415 mn using a microplate reader. The total phenolic content is shown as quercetin equivalents (mg QE/g extract) (Chimpalee et al., 2024).

2.3.3 Total chlorophyll content

The sample (10 mg/ml in MeOH) 200 µl was added into 98 well plate. The absorbance was measured at 645 and 663 nm by microplate reader. Total chlorophyll, chlorophyll-a and -b contents were calculated by equation (1), (2) and (3), respectively, in mg per g extract unit (Arnon, 1949).

$$\text{Total chlorophyll content (mg/g extract)} = [(20.2 \times A_{645}) + (8.02 \times A_{663})] / (1000 \times W) \quad (1)$$

$$\text{Chlorophyll-a content (mg/g extract)} = [(12.7 \times A_{663}) - (2.69 \times A_{645})] / (1000 \times W) \quad (2)$$

$$\text{Chlorophyll-b content (mg/g extract)} = [(22.9 \times A_{645}) - (4.68 \times A_{663})] / (1000 \times W) \quad (3)$$

where w is sample weight (g)

2.3.4 Total Carotenoid content

The sample (10 mg/ml in MeOH) 200 µl was added into 98 well plate. The absorbance was measured at 480 and 510 nm by microplate reader. Carotenoid content was calculated by equation (4) in mg per g extract unit (Momin and Kadam, 2011).

$$\text{Carotenoid content (mg/g extract)} = [(7.6 \times A480) - (1.49 \times A510)] / (1000 \times W) \quad (4)$$

where w is sample weight (g)

2.4 Statistical analysis

The results were presented in mean±SD. The t-test was used to compare the mean between the sample and the positive control using SPSS software.

3. Results

3.1 Antioxidant activities

The result of Sanan Tripop oil recipe showed the %inhibition in a concentration dependent manner (Figure 1). However, Sanan Tripop oil recipe has a less antioxidant activity IC_{50} value of 825.04±24.65 µg/ml in ABTS assay and it's showed no antioxidant activity (IC_{50} >1000 µg/ml) in DPPH assay (Table 1). However, the Sanan Tripop oil recipe showed the DPPH and ABTS values of 11.23±0.18 and 20.53±0.40 mg TE/g extract, respectively. Their values were calculated from Trolox standard curve from DPPH assay ($y = 0.0413x - 0.0127$; $R^2 = 0.9969$) and ABTS assay ($y = 0.052x + 0.0132$; $R^2 = 0.9993$). For FRAP assay, the Sanan Tripop oil recipe showed low value of 13.24±0.78 mmol Fe^{2+}/100 g extract in Table 1.

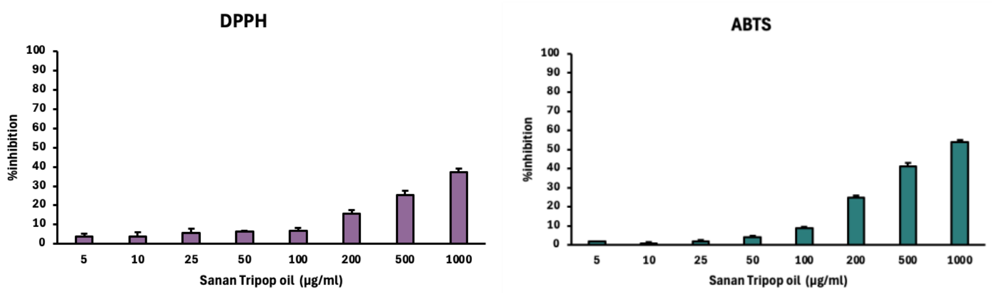

Figure 1. Antioxidant activities of Sanan Tripop oil recipe using DPPH and ABTS assays

Table 1. Antioxidant activities of Sanan Tripop oil recipe

Antioxidant activities		Sanan Tripop oil	Trolox
DPPH	IC_{50} (µg/ml)	>1000	7.46±0.05*
	DPPH value (mg TE/g extract)	11.23±0.18	-
ABTS	IC_{50} (µg/ml)	825.04±24.65	7.01±0.08*
	ABTS value (mg TE/g extract)	20.53±0.40	-
FRAP (mmol Fe^{2+}/100 g extract)		13.24±0.78	1240.70±11.37*

*Note: The * indicates the significance at $p<0.05$ using t-test.*

3.2 Chemical contents

The Sanan Tripop oil recipe demonstrated the total phenolic content of from the gallic acid standard curve ($y = 0.0647x - 0.0941$; $R^2 = 0.9915$) to obtain TPC value of 29.38±1.15 mg GAE/g extract. Total flavonoid content was calculated from quercetin standard curve ($y = 0.1309x + 0.0035$; $R^2 = 0.9993$) to obtain TFC value of 90.40±0.54 mg QE/g extract. In addition, the Sanan Tripop oil recipe showed the total chlorophyll and carotenoid contents in values of 0.08±0.00 and 0.03±0.00 mg/g extract, respectively (Table 2).

Table 2. Chemical contents of Sanan Tripop oil recipe

Chemical contents	Sanan Tripop oil
Total phenolic content (mg GAE/g extract)	29.38±1.15
Total flavonoid content (mg QE/g extract)	90.40±0.54
Total chlorophyll content (mg/g extract)	0.08±0.00
Total carotenoid content (mg/g extract)	0.03±0.00

4. Discussions

The Sanan Tripop oil recipe exhibited antioxidant capacity, although at a relatively low level. The ABTS assay revealed moderate antioxidant activity with an IC_{50} value of 825.04 ± 24.65 µg/mL and low ability to reduce iron of FRAP assay, while the DPPH assay showed no significant antioxidant activity. Interestingly, the Sanan Tripop oil recipe demonstrated antioxidant effects in a concentration-dependent manner, suggesting that its active constituents may exert biological activity at higher concentrations. These findings indicate that the formulation has potential for antioxidant activity, consistent with reports of other traditional medicinal plant extracts. Previous studies have also highlighted the antioxidant and anti-inflammatory effects of medicinal plants, supporting the relevance of this recipe for therapeutic applications (Kaźmierczak-Barańska et al., 2020).

The phytochemical analysis of the Sanan Tripop oil recipe showed moderate levels of bioactive compounds, including TPC value of 29.38 to 1.15 mg GAE/g extract, TFC value of 90.40 to 0.54 mg QE/g extract, including, Total chlorophyll content and Total carotenoid content value of 0.08 and 0.03 mg/g extract, respectively. These results also demonstrated a concentration-dependent trend. The presence of phenolics, flavonoids, and other phytochemicals is consistent with reports of secondary metabolites from medicinal plants, which are known to exert beneficial pharmacological effects (Wu et al., 2023). Importantly, phenolic compounds are strongly correlated with antioxidant activity in plants (Yongram et al., 2019), flavonoids have demonstrated strong radical-scavenging properties and chlorophylls have also been reported to exhibit antioxidant activity (Pérez-Gálvez et al., 2020).

5. Conclusion

The study demonstrated that the Sanan Tripop oil recipe exhibited weak antioxidant activity, with no detectable effect in the DPPH assay (IC_{50} >1000 μg/mL) and relatively low activity in the ABTS assay (IC_{50} = 825.04±24.65 μg/mL) and FRAP value of 13.24±0.78 mmol Fe^{2+}/100 g extract. Despite this limited antioxidant capacity, chemical analysis revealed that the formulation contains considerable levels of phenolics (29.38±1.15 mg GAE/g extract) and flavonoids (90.40±0.54 mg QE/g extract), along with measurable amounts of chlorophylls and carotenoids. These findings suggest that while the oil recipe does not exert strong free radical scavenging effects, its phytochemical profile may contribute to other potential bioactivities. Further studies, particularly focusing on pharmacological mechanisms and in vivo models, are recommended to clarify the therapeutic implications of these constituents.

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