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Effect of Different Solvent on Total Phenolic and Total Flavonoid Content of Sambang Colok Leaves extract (*Iresinediffusa*f. herbstii H)

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ABSTRACT: This study aimed to determine the total phenolic content (TPC) and total flavonoid content (TFC) of sambang colok (*Iresine diffusa*f. herbstii H) leaf extracts prepared using solvents with different polarities and to determine whether the solvent type affected the measured contents. Dried sambang colok leaf powder was extracted by Ultrasound Assisted Extraction (UAE) for 30 minutes using 96% ethanol, ethyl acetate, and n-hexane at a 1:10 sample-to-solvent ratio. Each extraction was performed in three replications. The extracts were subjected to phytochemical screening and thin-layer chromatography (TLC). Total phenolic content was determined by the Folin-Ciocalteu method using gallic acid as the standard, whereas total flavonoid content was determined by the AlCl3 method using quercetin as the standard. Absorbance was measured by UV-Vis spectrophotometry at 707 nm for TPC and 424.5 nm for TFC. Statistical analysis was performed using SPSS 25. The extracts were positive for phenolic, flavonoid, alkaloid, saponin, and steroid compounds. The highest TPC was obtained from the ethyl acetate extract, 42.5336 ± 1.6899 mg GAE/g, while the highest TFC was obtained from the ethanol extract, 38.2577 ± 5.2808 mg QE/g. Statistical analysis showed no significant difference among the three solvents for TPC (Kruskal-Wallis, p=0.061) or TFC (one-way ANOVA, p=0.054). Thus, although ethyl acetate and ethanol produced the highest numerical values for TPC and TFC, respectively, the solvent type did not significantly affect either parameter under the conditions of this study.

KEYWORDS: Sambang colok; solvent; Ultrasound Assisted Extraction; total phenolic content; total flavonoid content.

I. INTRODUCTION

[1–3]. Indonesia has abundant biological resources, including medicinal plants that have long been used in traditional medicine. Sambang colok (*Iresine diffusa*f. herbstii H) is one of the plants used empirically for several traditional purposes. The plant contains secondary metabolites such as flavonoids, polyphenols, saponins, tannins, and essential oils, while previous studies have reported pharmacological activities including anti-inflammatory, anticancer, analgesic, antidiabetic, and antipyretic effects.

[4–6]. Flavonoids are phenolic compounds rich in hydroxyl groups and are known for antioxidant activity through free-radical scavenging. Phenolic compounds are also natural antioxidants and may show antibacterial, antiseptic, and other biological activities. The extraction process is important for obtaining these compounds because the yield and composition of an extract depend on the extraction method and solvent used. Solvent polarity affects the compounds that can be dissolved according to the principle of like dissolves like.

[7,8]. Previous studies on other medicinal plants have shown that changing the extraction solvent can alter total phenolic and flavonoid contents. For example, different solvents produced different flavonoid levels in rambai laut leaves, while studies on white radish tubers also reported variation in phenolic and flavonoid contents according to solvent type. However, based on the KTI source, research specifically evaluating the effect of solvent type on total phenolic and total flavonoid contents of sambang colok leaves had not been identified. Therefore, this study evaluated 96% ethanol, ethyl acetate, and n-hexane using UAE and determined TPC and TFC of the resulting extracts.

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II. MATERIALS AND METHODS

The study used a true experimental design. Sambang colok leaf simplicia was obtained from the Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT), Tawangmangu, Karanganyar, Central Java, and the plant identity was determined at the same institution, currently referred to as UPF Pelayanan Kesehatan Tradisional RSUP Dr. Sardjito. Simple random sampling was used. The independent variable was extraction solvent (96% ethanol, ethyl acetate, and n-hexane), while the dependent variables were total phenolic and total flavonoid contents.

For each replication, 50 g of dried leaf powder was extracted with solvent at a 1:10 ratio using Ultrasound Assisted Extraction (UAE) for 30 minutes. The filtrate was concentrated in a water bath at 50°C and the concentrated extract was stored under refrigerated conditions. Extraction was performed in three replications for each solvent. Phytochemical screening was conducted for alkaloids, flavonoids, phenolics, saponins, and steroids/terpenoids, followed by TLC confirmation.

Total phenolic content was determined using the Folin-Ciocalteu method with gallic acid as the reference standard. Gallic acid standard solutions were prepared at 50, 60, 70, 80, 90, and 100 ppm. The maximum wavelength used was 707 nm and the operating time was 30 minutes. Results were expressed as mg gallic acid equivalent per gram of sample (mg GAE/g). Total flavonoid content was determined using the AlCl3 method with quercetin as the reference standard. Quercetin standards were prepared at 40, 50, 60, 70, 80, and 90 ppm. The maximum wavelength was 424.5 nm and the operating time was 10 minutes. Results were expressed as mg quercetin equivalent per gram of sample (mg QE/g).

Data were analyzed using SPSS version 25. Normality and homogeneity tests were performed first. Because the TPC data were normally distributed but not homogeneous, the Kruskal-Wallis test was used. TFC data were normally distributed and homogeneous, so one-way ANOVA was used. A p-value greater than 0.05 was interpreted as indicating no statistically significant difference.

III. RESULTS AND DISCUSSION

The extraction results showed that the highest mean yield was obtained from 96% ethanol (15.28 ± 4.1290%), followed by n-hexane (10.11 ± 1.0983%) and ethyl acetate (5.56 ± 4.6151%). The ethanol result is consistent with the greater polarity of ethanol and its ability to dissolve a broader range of polar constituents. Although the replicate yields varied, statistical analysis in the KTI showed no significant difference among the replicate yield data (p=0.095).

Table 1. Extraction yield of sambang colok leaf extracts.

Solvent	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)
96% Ethanol	9.93	19.98	15.94
Ethyl acetate	2.12	2.49	12.09
n-Hexane	11.45	10.14	8.76

Phytochemical screening showed that all three extracts were positive for phenolic, flavonoid, alkaloid, saponin, and steroid compounds. TLC confirmation also supported the presence of these five groups. Alkaloid identification used chloroform:ethyl acetate (70:30), while flavonoid identification used n-hexane:glacial acetic acid:water (4:1:5). Phenolics were identified using ethyl acetate:methanol:water (100:13.5:10), saponins using chloroform:methanol:water (64:50:10), and steroids using n-hexane:ethyl acetate (17:3). The TLC observations showed positive spots across the three extracts, with differences in Rf values reflecting differences in compound polarity and composition.

The total phenolic content is presented in Table 2. The ethyl acetate extract produced the highest mean TPC, followed by ethanol and n-hexane. Ethyl acetate is a semipolar solvent and, under the conditions of this study, was able to extract phenolic compounds more effectively than the other solvents. This finding is in agreement with the cited study of ethyl acetate fractions of Dayak onion leaves, which reported a higher total phenolic content in the ethyl acetate fraction than in the ethanol extract [9].

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Table 2. Total phenolic and total flavonoid contents of sambang colok leaf extracts.

Solvent	TPC (mg GAE/g)	TFC (mg QE/g)	Statistical result
96% Ethanol	22.7305 ± 5.2453	38.2577 ± 5.2808	TPC p=0.061; TFC p=0.054
Ethyl acetate	42.5336 ± 1.6899	26.4857 ± 2.7460	No significant difference
n-Hexane	17.6538 ± 0.5851	32.9642 ± 5.2714	No significant difference

The TPC data had Shapiro-Wilk significance values of 0.064, 0.728, and 0.987 for ethanol, ethyl acetate, and n-hexane, respectively, indicating normal distributions. However, the homogeneity test gave p=0.027, so the data were not homogeneous. The Kruskal-Wallis test produced p=0.061 (>0.05), indicating no significant difference in TPC among the three solvents.

For TFC, ethanol produced the highest mean value (38.2577 ± 5.2808 mg QE/g), followed by n-hexane (32.9642 ± 5.2714 mg QE/g) and ethyl acetate (26.4857 ± 2.7460 mg QE/g). The result is consistent with the cited work showing that ethanol can be effective in extracting flavonoids from plant material [10]. The normality results for ethanol, ethyl acetate, and n-hexane were p=0.324, 0.575, and 0.281, respectively, while the homogeneity test gave p=0.329. One-way ANOVA gave p=0.054 (>0.05), showing no statistically significant difference in TFC among the solvents.

Overall, the numerical differences in TPC and TFC demonstrate that solvent polarity can influence extraction performance, but the statistical tests in this study did not demonstrate a significant effect of solvent type on either parameter. Thus, the highest numerical TPC was obtained with ethyl acetate, while the highest numerical TFC was obtained with ethanol.

IV. CONCLUSION

The total phenolic contents of sambang colok leaf extracts were 22.7305 ± 5.2453 mg GAE/g for 96% ethanol, 42.5336 ± 1.6899 mg GAE/g for ethyl acetate, and 17.6538 ± 0.5851 mg GAE/g for n-hexane. The total flavonoid contents were 38.2577 ± 5.2808 mg QE/g, 26.4857 ± 2.7460 mg QE/g, and 32.9642 ± 5.2714 mg QE/g, respectively. Statistical analysis showed no significant difference among solvent types for TPC (p=0.061) or TFC (p=0.054). Ethyl acetate gave the highest numerical TPC, whereas 96% ethanol gave the highest numerical TFC. Further research is recommended to evaluate antioxidant activity and to use more specific analytical techniques such as LC-MS for identification and quantification of active compounds.

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