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Effect of drying methods on total flavonoid content of *Scurrula ferruginea* (Jack) Danser (*Loranthaceae*) leaf extracts parasitizing jengkol (*Pithecellobium jiringa*)

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ABSTRACT

Scurrula ferruginea (Benalu) is a hemiparasitic plant with potential as a raw material for traditional medicine due to its high flavonoid content. The concentration of flavonoids is substantially determined by the specific drying technique applied. Studies investigating the use of the stir-frying method remain limited, and there is no standardized protocol has yet been established to recommend an appropriate method for preserving the total flavonoid content in *S. ferruginea* leaves. This study aims to compare the total flavonoid content (TFC) in ethanol extracts of Benalu leaves on the Jengkol dried using three different methods: air-drying, oven-drying, and stir-frying drying. All samples were subsequently powdered and subjected to maceration using ethanol solvent, followed by analysis of their total flavonoid content using spectrophotometry UV-Vis at maximum wavelength at 431 nm. Statistical analysis of one-way ANOVA showed that different drying methods generated significant effects on TFC level of Benalu leaves extracts with significance value = 0.001 (p-value <= 0.05). The results indicated that air-drying yielded the highest flavonoid content with 7.37 ppm, followed by stir-frying (4.93 ppm) and oven-drying (2.88 ppm). These findings highlight the critical importance of selecting an appropriate drying method to preserve flavonoid levels, thereby enhancing the pharmacological efficacy and quality of herbal products derived from *S. ferruginea* leaves.

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1. INTRODUCTION

Scurrula ferruginea (Jack) Danser, commonly known as mistletoe, is a hemiparasitic plant widely utilized in traditional medicinal practices across Indonesia. The leaves of *S. ferruginea* have long been used as a primary raw material in various herbal formulations, particularly herbal teas, owing to their diverse secondary metabolite composition, which includes flavonoids, polyphenols, tannins, saponins, and steroids [1, 2]. Flavonoids are notably prominent in the pharmacological activity of mistletoe leaves, mostly due to their antioxidant and anti-inflammatory mechanisms [1]. However, as a parasitic plant, *S. ferruginea* obtains its nutrients by tapping into the physiological resources of its host. Hence, the host species exerts a significant influence on the chemical composition and biological activities of *S. ferruginea* [1, 3]. Several investigations have explored the phytochemical profiles of *S. ferruginea* parasitizing diverse host species; however, research addressing the secondary metabolite composition of *S. ferruginea* inhabiting *Pithecellobium jiringa* remains notably limited. Accordingly, a quantitative assessment of the total flavonoid content in *S. ferruginea* leaves associated with *P. jiringa* is warranted to elucidate the influence of host specificity on its phytochemical characteristics.

The efficacy of *S. ferruginea* leaves as a raw material for herbal products is significantly influenced by the processing of the crude drug, especially during the drying stage. The drying process aims to reduce the moisture content to below 10% of the initial weight, thereby inhibiting the growth of pathogenic microorganisms and extending the product's shelf life [4]. Furthermore, drying is essential for maintaining phytochemical constituents, especially flavonoids, which are thermolabile and prone to degradation as temperatures increases. Therefore, choosing an appropriate drying method is essential for preserving the quality of the crude medication and sustaining he pharmacological efficacy of the final herbal product.

Various drying methods have been utilized for *S. ferruginea*, encompassing air-drying, oven-drying, sun drying, shade drying, stir-frying, vacuum drying, and freeze-drying. Each drying approach presents distinct advantages and limitations in preserving flavonoid content. Air-drying is a widely traditional used technique due to its low risk of thermal degradation; however, it requires a relatively long duration and potentially carries a risk of enzymatic oxidation. In contrast, oven-drying accelerates the drying process; however, the high thermal exposure associated with this method may promote flavonoid degradation [5]. Stir-frying, a technique frequently utilized in traditional Chinese medicine preparation, can cause notable alterations in the chemical structure of flavonoids, involving both degradation and transformation reactions [6].

Previous investigations into *S. ferruginea* have largely centered on comparisons between air-drying and oven-drying techniques, primarily assessing their effects on total phenolic content and antioxidant activity [7]. Nevertheless, studies explicitly evaluating the impact of various drying approaches especially stir-frying—deeply rooted in local ethnopharmacological practice—on the total flavonoid content (TFC) of *S. ferruginea* leaves remain scarce. In addition, there is currently no standardized drying protocol aimed at optimizing flavonoid preservation in *S. ferruginea* leaf materials [7]. This culturally integrated approach not only bridges traditional knowledge and modern analytical evaluation but also provides novel insights into host-specific and thermally induced variations in flavonoid stability.

Accordingly, this study aims to evaluate and compare the total flavonoid content (TFC) of *S. ferruginea* leaf simplicia subjected to three drying methods: air-drying, oven-drying, and stir-frying. The findings are anticipated to provide evidence-based recommendations for the optimal drying method to preserve flavonoid integrity, supporting the development of high-quality herbal products derived from *S. ferruginea* leaves.

2. RESEARCH METHODS

2.1. Materials and Equipments

The materials used in this study included *Scurrula ferruginea* (Jack) Danser leaves parasitizing *Pithecellobium jiringa* (Jengkol tree), quercetin standard (Sigma-Aldrich), ethanol p.a. (Merck), aluminum chloride ($AlCl_3$), and potassium acetate (CH_3COOK). This study employed a UV–Visible spectrophotometer for quantitative analysis.

2.2. Sample Preparation

Leaves of *S. ferruginea* (Jack) Danser (*Loranthaceae*) parasitic on *Pithecellobium jiringa* were harvested from Lubuk Alung District, West Sumatra, Indonesia. Three drying approaches were applied to the collected material, namely air-drying (AD), oven-drying (OD), and stir-frying drying (SFD). In the air-drying technique, the leaves were allowed to dry naturally at room temperature under shaded conditions, avoiding direct exposure to sunlight, for a period of two weeks. For the oven-drying process, the samples were dehydrated in a drying oven maintained at 60°C for 48 hours. The stir-frying drying method involved heating the leaves over a moderate flame (approximately 180°C) for 10 minutes until a light brown coloration appeared, as adapted from the method described by Leng et al. (2017) [8]. Following the drying procedures, the samples were finely milled and stored in sealed containers prior to analysis.

2.3. Sample Extraction

Powdered leaf simplicia obtained from each drying treatment were subjected to maceration using ethanol as the extraction solvent. Approximately 200 g of dried powder was immersed in 1 L of

96% ethanol, maintaining a solid-to-solvent ratio of 1:5 (w/v). The maceration process was carried out for 72 hours at room temperature, with gentle agitation every 24 hours to enhance the extraction efficiency. After maceration, the filtrates were pooled and concentrated under reduced pressure using a rotary evaporator to remove the solvent. The semi-solid extracts were further thickened on a water bath to eliminate residual solvent, following the modified procedure [9]. The concentrated extracts were subsequently weighed, and the extraction yield was calculated as a percentage relative to the initial dry weight of the plant material.

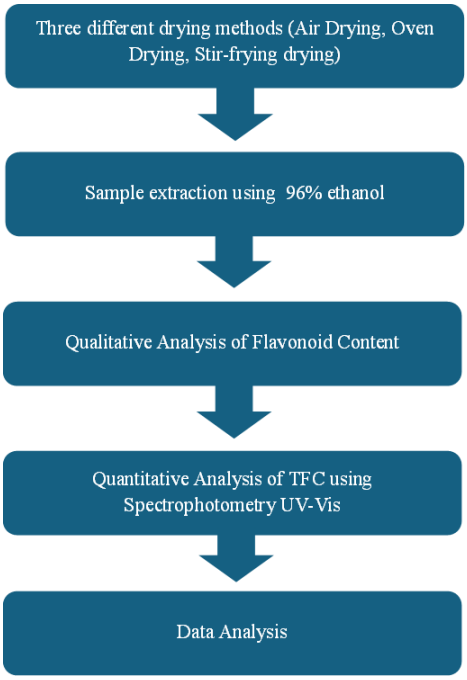

Figure 1. Research method flow chart.

2.4. Qualitative Analysis of Flavonoid

Test with Dilute Sodium Hydroxide (NaOH) Reagent.A portion of the extract was treated with a few drops of dilute NaOH solution. The appearance of a yellow coloration indicated a positive reaction, confirming the presence of flavonoid compounds in the sample.

Wilstätter Cyanidin Test, in this assay, a small amount of extract was mixed with magnesium powder, followed by the addition of concentrated hydrochloric acid (HCl p.a.). The formation of an orange to reddish hue signified a positive result, suggesting the presence of flavonoids within the tested extract.

2.5. Quantitative Determination of Total Flavonoid Content Using UV-Visible Spectrophotometry

2.5.1. Preparation of Quercetin Standard Solution and Calibration Curve

A quercetin standard stock solution (1000 ppm) was prepared by accurately weighing 25 mg of quercetin reference standard (Sigma-Aldrich) and dissolving it in ethanol p.a. to a final volume of 25 mL in a volumetric flask. The maximum absorbance wavelength (λ_{max}) of quercetin was determined by scanning the standard solution within the wavelength range of 400–500 nm using a UV–Visible spectrophotometer, and the wavelength corresponding to peak absorbance was selected for further analysis. From the stock solution, a 100 ppm working solution was obtained by diluting 1 mL of the 1000 ppm stock to 10 mL with ethanol. This working solution was subsequently diluted to prepare standard concentrations of 6, 8, 10, 12, and 14 ppm. For color complex formation, 1 mL of each standard was mixed with 1 mL of 2% $AlCl_3$ and 1 mL of 0.12 M potassium acetate. The mixtures were allowed to react for one hour at room temperature, after which the absorbance was measured at the determined λ_{max} using a UV–Visible spectrophotometer [10]. The calibration curve was plotted using the resulting absorbance values against concentration.

2.5.2. Sample Preparation and Measurement

For sample analysis, 15 mg of the ethanol extract was dissolved in 10 mL of ethanol to obtain a 1500 ppm solution. A 1 mL aliquot of this solution was combined with 1 mL of 2% $AlCl_3$ and 1 mL of 0.12 M potassium acetate. The reaction mixture was incubated for one hour at room temperature to allow complex formation, after which absorbance was recorded at 431 nm. Each sample was analyzed in triplicate, and the mean absorbance value was used for quantification [10].

2.5.3. Data Analysis

The total flavonoid content (TFC) of each extract was calculated based on the quercetin calibration curve using the regression equation $y = 0.1207x + 0.3973$ ($R^2 = 0.9965$). The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

3. RESULTS AND DISCUSSIONS

Three different drying techniques—air-drying (AD), oven-drying (OD), and stir-fry drying (SFD)—were employed for the preparation of *S. ferruginea* (Jack) Danser simplicia. Based on the color analysis of the powdered dried samples, all methods produced a green coloration similar to that of the fresh leaves. However, the sample obtained through the stir-fry drying process exhibited a darker greenish-brown shade compared to the other two drying techniques.

3.1. Effect of Drying Methods on the Physical Characteristics and Yield of *Scurrula ferruginea* Leaf Extract

Ethanolic extracts from all three drying methods produced thick, viscous extracts with a characteristic herbal odor, though noticeable variations in extract color were observed among the samples. The percentage yield of each extract is presented in Table 1.

Table 1. The percentage yield of Benalu leaf extract.

Drying methods	Yield (%)	Organoleptic properties
Air-drying	5.4	Thick texture, characteristic odor, deep green color
Oven-drying	6.2	Thick texture, characteristic odor, dark green color
Stir-fry drying	7.6	Thick texture, characteristic odor, dark greenish-brown color

As presented in Table 1, the stir-fry drying method produced the highest extract yield (7.6%), followed by oven-drying (6.2%) and air-drying (5.4%). These yield differences are primarily associated with the moisture content retained in the dried plant materials. The air-drying technique, conducted at ambient temperature (27°C – 30°C), requires a prolonged drying period and tends to be less efficient in eliminating moisture compared with heat-assisted methods. As a result, this process often leaves a relatively high-water content, typically exceeding 10%, which can affect extraction efficiency [11]. Furthermore, the low temperature employed during air-drying may not adequately disrupt leaf cell structures, thereby limiting solvent penetration and reducing the release of intracellular metabolites. Conversely, the stir-fry drying technique, which exposes the samples to higher temperatures (approximately 180°C), promotes rapid water evaporation and enhances the breakdown of plant cell walls. This structural disruption facilitates the diffusion of bioactive compounds into the extraction solvent, resulting in higher extract recovery. Elevated drying temperatures have been reported to accelerate water loss and improve drying kinetics by increasing transpiration and evaporation rates in plant materials [12].

3.2. Qualitative Analysis of Flavonoid

The qualitative identification of flavonoids was performed using the NaOH reagent test and the Shinoda test (Mg + concentrated HCl). The results of the screening are summarized in Table 2. All extracts derived from the three drying techniques—air-drying (AD), oven-drying (OD), and stir-fry drying (SFD)—tested positive for flavonoids in both assays. A yellow coloration in the NaOH test and a red to reddish-brown hue in the Shinoda reaction confirmed the presence of flavonoid compounds in each extract.

Tabel 2. Qualitative analysis of flavonoid in Benalu leaf extracts.

Reagent	Color change	Test results		
		AD	OD	SFD
HCl p.a + Mg	Red/reddish brown	+	+	+
NaOH	Yellow	+	+	+

Note: • (+) Indicates the presence of flavonoid
• AD: Air-drying; OD: Oven-drying; SFD: Stir-frying drying

3.3. Quantitative Determination of TFC

The total flavonoid content (TFC) of S. ferruginea leaf extracts was quantified using a UV–Visible spectrophotometric method. This technique was selected due to the ability of conjugated aromatic systems within flavonoids to absorb strongly in the ultraviolet and visible regions of the spectrum [13]. The maximum absorbance (λ_{max}) of the quercetin standard solution was observed at 431nm, and the calibration curve demonstrated a strong linear relationship between absorbance and concentration, as shown in Figure 2.

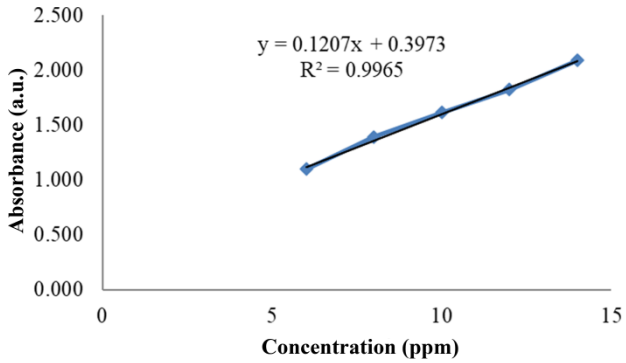

Figure 2. The quercetin calibration curve.

Based on the calibration curve, the TFC values of the extracts obtained from the three drying methods are presented in Table 3.

Table 3. Total flavonoid content (TFC) of *S. ferruginea* leaf extracts prepared using different drying methods.

Drying methods	Replicate	Absorbance	TFC (ppm)
Air-drying	1	1.267	7.205
	2	1.314	7.595
	3	1.280	7.313
Oven-drying	1	0.698	2.491
	2	0.746	2.889
	3	0.791	3.262
Stir-fry drying	1	0.970	4.745
	2	1.015	5.118
	3	0.993	4.935

Normality testing using the Shapiro–Wilk method confirmed that the data were normally distributed ($p > 0.05$), as shown in Table 4.

Table 4. Saphiro Wilk normality test results.

Drying method	Df	Sig
AD	3	0.519
OD	3	0.964
SFD	3	0.979

A one-way ANOVA performed at a 95% confidence level indicated a statistically significant effect of the drying method on the mean TFC values 0.001 ($p < 0.05$). As presented in Figure 3, the TFC content varied between 2.88 ppm and 7.37 ppm. Among the three drying approaches, air-drying produced the highest TFC (7.37 ppm), followed by stir-fry drying (4.93 ppm) and oven-drying (2.88 ppm).

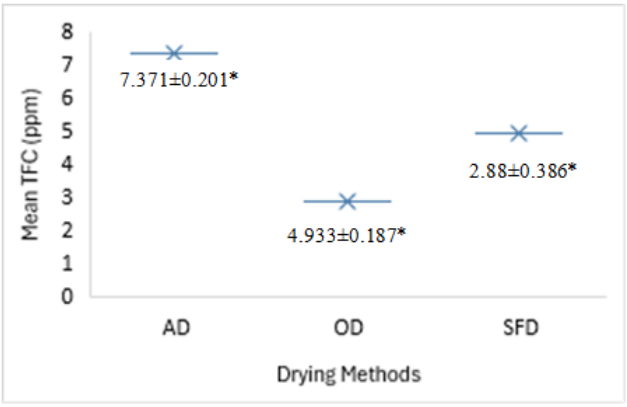

Figure 3. Effect of drying methods on the total flavonoid content (TFC) of *S. ferruginea* leaf extracts. Values are presented as mean ± SD (n = 3). The asterisk (*) denotes a statistically significant difference ($p < 0.05$).

3.4. Discussion

The present findings align with previous reports showing that air-drying tends to preserve higher flavonoid levels than thermal drying methods. Previous study reported that ethanolic extracts of *Coleus amboinicus* leaves processed by air-drying exhibited higher TFC (5.83 mg QE/g extract) compared with oven-dried samples (3.58 mg QE/g extract at 50°C) [14]. Similarly, earlier finding found that acetone extracts of *S. ferruginea* obtained from air-dried samples contained higher flavonoid levels than those dried in an oven [7]. However, these previous study have not evaluated the effects of traditional stir-frying—a culturally embedded preparation method in local ethnomedicinal practice—on the flavonoid stability of *S. ferruginea*.

Flavonoids are known to be thermolabile compounds that readily degrade at elevated temperatures. Prolonged exposure to heat during drying can trigger enzymatic oxidation and thermal decomposition, leading to the loss of volatile phytochemicals [15]. Kankara et al. (2014) also observed a marked reduction in TFC in Guiera senegalensis leaves following oven drying at 75°C for 12 h [16]. Likewise, Susiani et al. (2017) reported a progressive decline in TFC of *Orthosiphon aristatus* extracts as the oven temperature increased from 30°C to 70°C [17].

Quercetin, quercitrin, and 4″-O-acetylquercitrin have been identified in the leaf extracts but are scarcely detected in the stems of *S. ferruginea* [18]. Prolonged exposure to elevated temperature resulted in the progressive degradation of quercetin, with the initial degradation detected at approximately 17.57 minutes and a half-life estimated at 169.72 minutes [19]. This thermal instability is closely associated with the compound's molecular structure. The presence of two hydroxyl substituents on the B-ring of quercetin increases its susceptibility to oxidative degradation, as B-ring hydroxylation facilitates oxidation and structural rearrangement under heat stress. In contrast, galangin exhibits higher thermal stability among flavonol analogues, attributed to the absence of hydroxyl groups on its B-ring [20].

The darkening of *S. ferruginea* leaves under thermal drying conditions likely reflects oxidative polymerization of flavonoid and phenolic compounds. Comparable findings in other plant matrices have demonstrated that reduced color brightness coincides with lower flavonoid concentrations, indicating that pigment formation parallels oxidative degradation processes [4]. These results suggest that intensified heat exposure promotes polymerization and condensation of oxidized intermediates, which both diminish flavonoid stability and contribute to visible color alteration.

Interestingly, in the present study, the stir-fry drying method yielded a higher TFC (4.93 ppm) than oven drying (2.88 ppm). Although stir-fry drying involves a higher temperature (approximately 180°C), the short exposure time (10 min) likely minimized oxidative degradation. In contrast, oven

drying at 60°C for 48 h, despite using a lower temperature, may have promoted greater flavonoid loss due to extended heat exposure. This result supports the notion that both temperature and drying duration jointly influence flavonoid stability [21]. The stir-frying process in Citrus reticulata 'Chachi' influenced flavonoid profiles such as hesperidin, nobiletin, and tangeretin [22]. Concentrations of these compounds increased during the initial heating phase but declined with prolonged exposure, suggesting that an optimal heating duration is critical for minimizing degradation.

Although oven drying provides consistent temperature control (60°C), its extended duration (48 h) increases the risk of flavonoid degradation. As noted by [11], hot-air drying at 60°C for 6–8 h effectively reduces moisture levels compared with sun drying; however, longer drying times may promote water–flavonoid co-evaporation due to their water-soluble nature. Consequently, prolonged oven drying may result in the lowest flavonoid recovery among the tested methods.

This study clearly demonstrates that the drying technique employed exerts a substantial impact on the flavonoid content of ethanolic leaf extracts of *Scurrula ferruginea* parasitizing *Pithecellobium jiringa*. These findings provide valuable insights for practical applications in herbal product manufacturing by identifying the most effective drying method to maximize the total flavonoid content in *S. ferruginea* leaf extracts.

In addition to its practical implications for herbal standardization, this study also contributes to the concept of sustainable and green processing. The identification of air-drying as the most effective method for preserving flavonoid content, followed by traditional stir-frying and oven drying, highlights the potential of low-energy and culturally rooted techniques in achieving both phytochemical efficiency and environmental sustainability. These findings may therefore inform the development of eco-friendly processing guidelines for medicinal plants in Indonesia.

However, it should be acknowledged that the present study did not include an evaluation of post-extraction antioxidant activity, which would have provided additional insight into the biological significance of the observed TFC values. Future studies integrating phytochemical profiling with functional bioassays are therefore warranted to establish a more comprehensive understanding of the pharmacological potential of S. ferruginea extracts.

4. CONCLUSION

In summary, this study successfully investigated the influence of different drying methods on the total flavonoid content (TFC) of ethanol extracts derived from Scurrula ferruginea (Jack) Danser leaves parasitizing Pithecellobium Jiringa (jengkol). The TFC of the extracts was evaluated following three distinct drying techniques: air-drying (approximately 27 °C for two weeks), oven-drying (60 °C for 48 hours), and stir-frying drying (180 °C for 10 minutes). The findings demonstrated that the air-drying method yielded the highest average TFC (7.37 ppm), followed by stir-fry drying (4.93 ppm) and oven-drying (2.88 ppm). Statistical analysis confirmed that the differences among drying methods were significant ($p \leq 0.05$). These results highlight the critical role of drying techniques in preserving flavonoid content and ensuring the production of high-quality, pharmacologically effective herbal materials. Future research should further explore the impact of various drying methods on other bioactive constituents—such as polyphenols, tannins, and saponins—to gain a more comprehensive understanding of the phytochemical quality of S. ferruginea leaf simplicia. Additionally, extended studies assessing how drying methods affect the extract's pharmacological properties, including antioxidant and anti-inflammatory activities, as well as the long-term stability of its bioactive compounds, are recommended.

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