A preliminary study of phytochemical quantification and thermal characterization of *Faloak* (*Sterculia quadrifida* R. Br) leaf powder

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Abstract: Native to East Nusa Tenggara, Indonesia, the *faloak* plant is used in traditional medicine, concentrating on its bark. *Faloak* leaves, on the other hand, are a plentiful and promising natural resource complete with phytochemical components that are worth investigating for both food and medical uses. Thus, this work aims to determine the phytochemical quantification, represented by total phenolic and flavonoid content, and the thermal properties of *faloak* leaf powder originating from East Nusa Tenggara. The result revealed that the *faloak* leaf powder has a high total phenolic and flavonoid content of 89.26 mg GAE/g DW and 9.57 mg QE/g DW, respectively. *Faloak* leaf powder has a particle size distribution of 0.145 to 110.467 μm, with a d_{50} of 10.072 μm. Also, it has a negative surface charge, with a value of -19.1 mV, based on the zeta potential analysis. The presence of phenolic and flavonoid compounds in *faloak* leaf powder was confirmed by the fingerprint chemical bounds of those compounds in Fourier transform infrared (FTIR) spectra. Furthermore, thermogravimetric analysis (TGA) and differential thermal analysis (DTA) demonstrated that the *faloak* leaf powder revealed good stability at moderate temperatures. As a result, *faloak* leaf powder has the potential and promise to be used as a functional ingredient in developing innovative functional food products or applications in other fields, such as pharmaceuticals.

Keywords: characterization, *faloak* leaves, phytochemical, thermal analysis

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1 Introduction

The *faloak* plant is a native Indonesian medicinal plant that is found wild, mainly in the East Nusa Tenggara plains, such as on Rote Island, Alor Island, and Timor Island, and is locally known as "*Flolo"* (Tenda et al., 2019). In East Nusa Tenggara, the local people commonly use the *faloak* plant parts, especially the *faloak* bark, as a traditional medicine

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passed down from generation to generation, and is still preserved until now, with the simple and traditional processing method, which is only boiling *faloak* bark in water to obtained water extract of *faloak* bark. Furthermore, the water extract of *faloak* bark is usually consumed to treat internal diseases, such as helping to treat typhoid, ulcers, and liver disease, increase stamina, and reduce fatigue (Siswadi et al., 2015, 2021; Siswadi and Saragih, 2021). Yet, until nowadays, the exploration and utilization of *faloak* plants have only focused on *faloak* bark (Ruskim et al., 2023; Siswadi et al., 2015, 2013). Nevertheless, the *faloak* leaves are promising natural resources and abundance to explore for medicinal and food purposes. The *faloak* leaves are rich in phytochemicals like flavonoids, steroids, terpenoids, and tannins (Akter et al., 2016). Based on the study by Rollando et al. (2022), the *faloak* leaves may be cytotoxic to breast cancer cells. Additionally, the Aboriginal people in Australia used the *faloak* leaves applied topically to stings, wounds, blisters, and skin issues (Akter et al., 2016). However, the phytochemical quantification, especially for the total phenolic and flavonoid content, and the thermal characterization of *faloak* leaf powder have not been rarely widely explored, and it is interesting to uncover and learn more, especially those from the native region of East Nusa Tenggara, Indonesia.

The unveiling of the phytochemical compound content and thermal properties of *faloak* leaf powder is one of the essential steps to identify and further explore its potential for developing new functional food and beverage products or further utilization for related fields such as pharmaceuticals and other fields. Fourier transform infrared (FTIR) is a commonly used method to identify phytochemical compounds in plant powders and extracts qualitatively. FTIR is a technique that uses infrared radiation beams to identify functional groups in gaseous, liquid, and solid materials (Khan et al., 2018) and has been successfully applied previously to identify biomolecules found in powders and extracts from *Enhalus acoroides* leaves (Pharmawati and Wrasiati, 2020). Besides that, several researchers have employed thermo-analytical methods to characterize powder plants (Jeyakumar et al., 2020; Manju et al., 2021; Russo et al., 2018). This technique has the advantages of being quick, requiring little sample, and potentially being more environmentally friendly than other approaches like high-performance liquid chromatography (HPLC), which takes time, necessitates multiple extraction steps using different polarity solvents, and creates residues that are harmful to the environment (Russo et al., 2018). Therefore, based on these facts, this study focuses on quantifying phytochemical compounds, represented by the total phenolic and flavonoid content, and the thermal characterization of *faloak* leaf powder originating from East Nusa Tenggara, Indonesia.

2 Materials and Methods

2.1 Materials

The mature *faloak* (*Sterculia quadrifida* R. Br**)** leaves were obtained from the Timor Tengah Utara Regency in East Nusa Tenggara Province, Indonesia. Then, the *faloak* leaves were dried in a cabinet dryer at 60°C until constant weight (± 8 h) and then ground using a blender and sieve to 100-mesh to obtain the *faloak* leaf powder. Then, the *faloak* leaf powder was transferred to Universitas Brawijaya, Malang, Indonesia, for further analysis. The analysis of *faloak* leaf powder was conducted at the Central Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia. The distillate water was purchased from a local chemical supplier in Malang City. The gallic acid and quercetin standards were purchased from Sigma-Aldrich (St. Louis, USA). Other chemical reagents, such as methanol, folin–ciocalteu reagent, sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3$), and sodium nitrite ($NaNO_2$), were provided by Merck (Darmstadt, Germany). All reagents used in this study were analytical grades.

2.2 Total phenolic and total flavonoid content analysis

The phenolic content of the *faloak* leaf powder

was determined using the method described by López et al. (2011). Briefly, *faloak* leaf powder was extracted using methanol as a solvent at a 1:5 w/v ratio for at least 60 min. Then, 0.5 ml of methanolic extract of *faloak* leaf powder was mixed with 2.5 ml of the 10% v/v follin reagent, and the mixture was incubated for five minutes. After adding 2 ml of Na_2CO_3 (7.5% w/v) in the sixth minute, the mixture was incubated for 30 minutes in a dark environment. Next, a UV-Vis Spectrophotometer (UV-1900i UV-vis spectrophotometer, Shimadzu, Japan) was used to measure absorbance at a maximum wavelength (743 nm). The standard curve of gallic acid was constructed from 50 to 200 μg ml^{-1} concentration to estimate the total phenolic in *faloak* leaf powder and expressed in mg GAE g^{-1}. For total flavonoid analysis, 1 ml of methanolic extract of *faloak* leaf powder and 4 ml of distilled water were combined at tube test and added 0.3 ml of 5% of $NaNO_2$ and incubated for 5 min. At 6 minutes, 0.3 ml of 10% $AlCl_3$ was added to the mixture and incubated for another 6 min. Then, add 2 ml of 1 M NaOH and distilled water until the volume is 10 mL and incubate again for 30 min at room temperature in dark conditions. The absorbance was measured with a UV-visible spectrophotometer (UV-1900i UV-vis spectrophotometer, Shimadzu, Japan) at a maximum wavelength (410 nm). The standard curve of quercetin was constructed from 20 to 100 μg ml^{-1} concentration to estimate the total flavonoid in *faloak* leaf powder and expressed in mg QE g^{-1} (Sahu and Saxena, 2013).

2.3 Particle size, functional group, zeta potential, and thermal analysis

The particle size and functional group of the faloak leaf powder were assessed using methods described by Witoyo et al. (2023). Shortly, the particle analysis was performed using the particle size analyzer (Shimadzu SALD-7500 nano, Japan), and the functional group was assessed using a Shimadzu IR Spirit-FTIR spectrophotometer (Shimadzu, Japan) at a wavenumber range of 4000-400 cm^{-1} with number of scans of 20 and resolution of 2 cm^{-1}. A Litesizer DLS 500 device (Anton Paar Brand) was used to evaluate the zeta potential of the faloak leaf powder using dynamic light scattering techniques using a modified method adopted from Afifah et al. (2021). Before the measurement, the faloak leaf powder was diluted with distilled water in a 1:10 w/v ratio. Additionally, the thermal analysis of faloak leaf powder, including thermogravimetric analysis (TGA) and differential thermal analysis (DTA), was performed using the DTG-60H (Shimadzu, Japan) by heating 5 to 15 mg of sample in air atmospheres between 20°C and 600°C at a 20°C min^{-1} of heat rate, following the methodology described by Da Costa et al. (2002).

2.4 Data analysis

The phytochemical quantification and thermal characterization data of *faloak* leaf powder were analyzed in descriptive, and the results were explained by comparing them with similar earlier studies reported.

3 Results and discussion

3.1 Total phenolic and total flavonoid content

The total phenolic and total flavonoid content of *faloak* leaf powder in this study was 89.26 mg GAE/g DW and 9.57 mg QE/g DW in sequence. The total phenolic and flavonoid found in this study were higher than those reported earlier by several researchers. Dillak et al. (2019) reported that the ethanolic extract of *faloak* leaves had a total phenol of 3.43 mg/g sample and a total flavonoid of 12.56 mg/g sample. Saragih and Siswadi (2019) also reported that the ethanolic extract of *faloak* leaves had a total phenol of 9.29 mg GAE g^{-1} and a total flavonoid of 0.58 mg QE g^{-1}. Moreover, the *S. quadrifida* leaf extract from Australia had a total phenolic content of 52.46 mg GAE/g plant extract and a total flavonoid of 70.5 mg CE/g plant extract (Akter et al., 2016). The difference in phenolic compounds in *faloak* leaf powder, including total phenolic and total flavonoid, might be affected by the different sampling locations, seasons, the solvent used for extraction, and the age of plant samples (Chaves et al., 2020; Fratianni et al., 2007; Medini et al., 2014; Saragih and Siswadi, 2019).

Moreover, the high polyphenol compound, represented by the total phenolic and flavonoid, indicates that the *faloak* leaf powder has the potential as a source of antioxidants and also has the potential to be applied in a food product for supplementation or fortification to produce functional food with high antioxidant, or applied in related fields, such as the development of plant-based supplement with high bioactive compounds.

3.2 Particle size distribution

The particle size distribution of *faloak* leaf powder ranged from 0.145 to 110.467 µm, as revealed in Figure 1. The d_{50} of *faloak* leaf powder was 10.07±0.01 µm. Moreover, the *faloak* leaf powder has mean and module particle sizes of 8.87±0.01 µm and 10.76±0.00 µm, respectively. The d_{50} of this study was bigger than the d_{50} of *Moringa* leaf powder in an earlier study, which had d_{50} of 0.72 to 3.76 µm (Sakr et al., 2024). However, this study's *faloak* leaf powder has a median similar to d_{50} to the green tea leaf milled using a ball mill at 30 min, with a d_{50} of 10.6 µm (Zhao et al., 2023). Moreover, *faloak* leaf powder had d_{10} and d_{90} of 1.60±0.00 µm and 38.72±0.13 µm, respectively. The d_{10} in this study was in the range of the d_{10} of *Moringa* leaf powder, which is 0.66 to 1.65 µm, but the d_{90} was higher than in an earlier study, which had d_{90} in the range of 0.79 to 8.59 µm (Sakr et al., 2024). However, the d_{10} and d_{90} in this study were smaller than the d_{10} of *Moringa* leaf powder reported by Huang et al. (2020). In their studies, Huang et al. (2020) reported that Moringa leaf powder had d_{10} from 5.1 to 13.2 µm and d_{90} from 65.4 to 319.3 µm. Furthermore, in the food system, the particle size of the ingredient is an essential factor that affects the physicochemical, sensory, and functional properties of the product, like food powder and flour, as well as derived processed products (Chitrakar et al., 2023; Li et al., 2023; Salari et al., 2024; Savlak et al., 2016). So, the particle size selection of ingredients should be considered to obtain suitable and desirable food products.

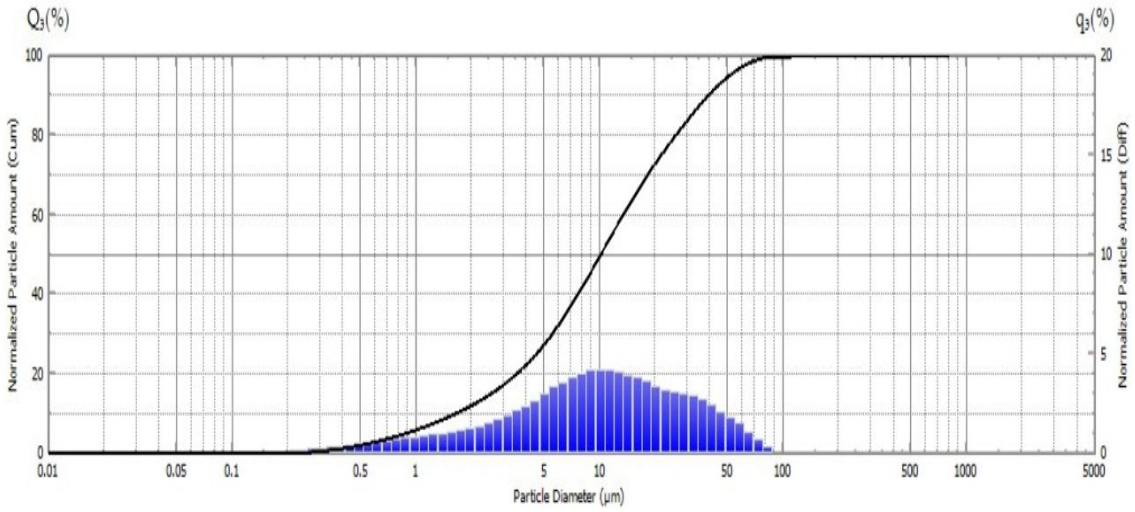

Figure 1 The particle size distribution of *faloak* leaf powder

3.3 Zeta potential

The *faloak* leaf powder has a negative surface charge, with a mean shape peak of -19.1±0.4 mV, based on the zeta potential value presented in Figure 2. This result was higher than the zeta potential of *Moringa* leaf extract, which was -23.07 mV (Wardana et al., 2022). However, these results had a similar zeta potential of betel leaf extract nanoparticles of -23.0 ± 0.35 mV (Saryanti et al., 2020). The nanoparticles with zeta potentials less than –25 mV and more than 25 mV had better stability (Afifah et al., 2021; Pandey et al., 2021; Saryanti et al., 2020; Wardana et al., 2022). So, this indicates that the *faloak* leaf powder has better stability in the solution. Whether positive or negative, the high zeta potential value suggests a significant electrostatic force to avoid phase separation (Saryanti et al., 2020). According to Afifah et al. (2021), the

high zeta potential value can prevent suspension particles from flocculating and aggregating.

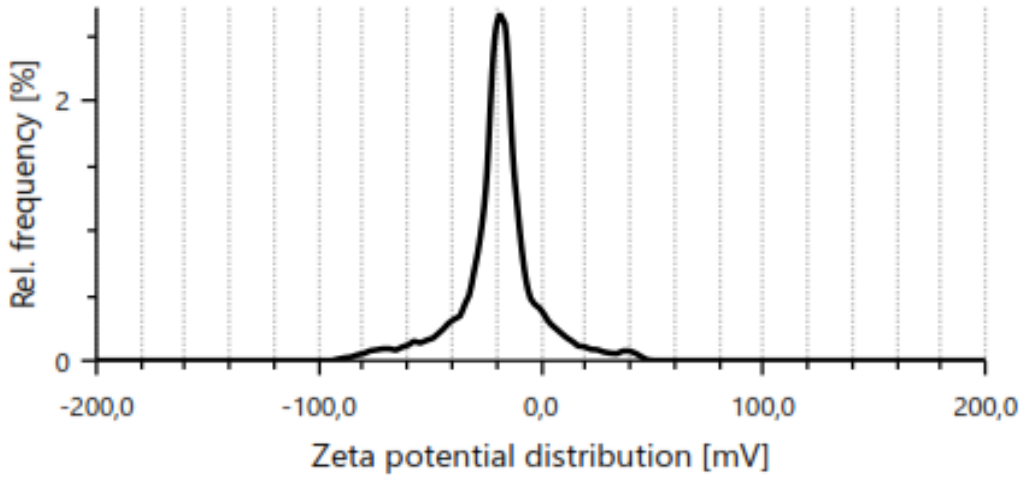

Figure 2 The zeta potential graph of *faloak* leaf powder

3.4 Functional group

The functional group of *faloak* leaf powder was assessed using FTIR, as exhibited in Figure 3 and listed in Table 1. Figure 3 shows that the O-H stretching bond in the *faloak* leaf was found at 3305.92 cm^{-1} (Nursanto et al., 2023; Putri et al., 2023). The C-H aliphatic bond was found at 2921.05 cm^{-1} and 2854.27 cm^{-1} (Putri et al., 2023). The wavenumbers at 1727.64 cm^{-1} and 1606.29 cm^{-1} were identified as C=O bonds in *faloak* leaf powder (Nursanto et al., 2023; Putri et al., 2023). Moreover, the C-C aromatic bond was found at a wavenumber of 1514.38 cm^{-1} and 1439.70 cm^{-1}, and the 1372.92 cm^{-1} and 1313.32 cm^{-1} were identified as O-H bending (Suzeta et al., 2023).

Another chemical bond also found in *faloak* leaf powder, like the C-O alcohol bond, was identified at a wavelength of 1246.54 cm^{-1} (Nursanto et al. 2023), and the peaks at a wavenumber of 1246.54 cm^{-1}, 1153.20 cm^{-1}, and 1034.72 cm^{-1} were identified as C-O ester bond (Suzeta et al., 2023). The peak at a wavenumber of 1034.72 cm^{-1} was also identified as a C-O-H bond (Putri et al., 2023). Furthermore, the 1246.54 cm^{-1}, 1153.20 cm^{-1}, and 1034.72 cm^{-1} were identified as C-N stretching (Putri et al., 2023; Suzeta et al., 2023). The C-H aromatics in *faloak* leaf powder were identified at 882.49 cm^{-1}, 820.74 cm^{-1}, 773.34 cm^{-1} (Nursanto et al., 2023), 556.49 cm^{-1}, and 509.82 cm^{-1} (Putri et al., 2023). Also, the C-OH aromatic bond was detected at a wavenumber of 432.27 cm^{-1} (Putri et al., 2023).

The phenolics compound fingerprint in *faloak* leaf powder was identified with O-H stretching, C-H aliphatic, C=O, C-C aromatic, O-H bending, C-O, C-N stretching, C-H aromatic, and C-OH aromatic bonds. Suzeta et al. (2023) reported that the peaks indicating phenolic compounds in soursop peel extract were C-N, C-O, C=C aromatic, C-H, and O-H groups. Furthermore, the *faloak* leaf powder also contained the flavonoid content indicating with the O-H stretching, C=O, O-H bending, C-O, C-O eter, and C-H aromatic. The primary functional groups in flavonoid compounds are found in three banana peel extracts: O-H, C=O, C=C aromatic, and C-H aromatic (Putri et al., 2023). Ekawati et al. (2017) also stated that the OH, C-H aliphatic, C-H aromatic, C=C aromatic, C-O alcohol, C=O, and C-O ether groups are the functional groups of flavonoid compounds. The chemical bonds of rearranged phenolic and flavonoid compounds in FTIR Spectra have supported the presence and the quantification of phenolic and flavonoid compounds in *faloak* leaf powder, as explained previously in section 3.1. Moreover, the other phytochemicals, like tannin, saponin, and alkaloids, are also found in *faloak* leaf powder, indicating the presence of chemical bonds for those compounds, as summarized in Table 1.

3.5 Thermal characterization

The thermal characterization of *faloak* leaf powder evaluated using TGA and DTA is revealed in Figure 4. The first weight loss of *faloak* leaf powder, around 8%, was found at 22.00°C to 241.20°C, indicating removing moisture content and light volatile substances (Reza et al., 2020a). da Costa et al. (2002) also reported that the first stage of degradation of pure quercetin in the air atmosphere occurs at 103°C to 342°C. Furthermore, in DTA curves, the water evaporation in the *faloak* leaf powder was also identified by a broad endothermic peak between 22.12°C and 156.01°C, and 84.62°C was the peak decomposition temperature and the volatile substances were identified in a peak between 154.44°C and 236.02°C. Khelloufi et al. (2017) reported that water evaporation in the shrub leaf was found at 50°C to 118°C. In another study, Malucelli et al. (2018) also reported that the 1st phase of degradation of *Dicksonia sellowiana* extract based on the DTA curve was marked by endothermic peaks with a peak temperature of 54.74°C. Jeyakumar et al. (2020) also found that the temperature around 171.96°C was attributed to the saponin breakdown.

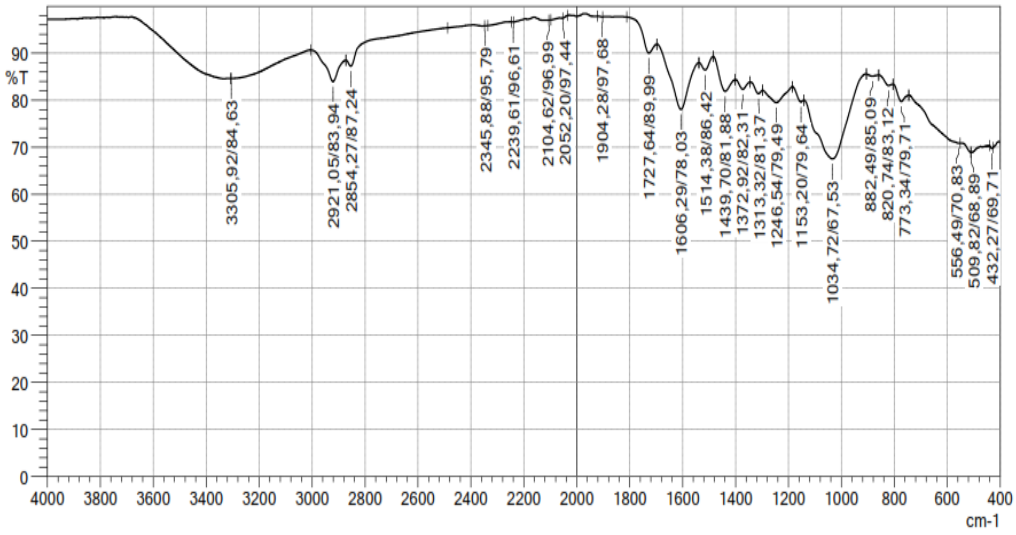

Figure 3 The FTIR spectra of *faloak* leaf powder at a wavenumber range of 4000 - 400 cm^{-1}

The second stages of weight loss and the significant weight degradation of *faloak* leaf powder were found at 241.13°C to 521.92°C, with a weight loss of around 76% based on TGA curves. The weight loss in this stage might be attributed to the degradation of the phytochemical compounds, including polyphenol compounds in *faloak* leaf powder. Several earlier studies reported the melting point of some phytochemical compounds like gallic acid, kaempferol, quercetin, and myricetin in *Moringa oleifera* powder, which occurs at temperatures of 258°C to 265°C, 277°C, 316.5°C, and 357°C, respectively (Jeyakumar et al., 2020; Manju et al., 2021; Tafu and Jideani, 2021). According to Da Costa et al. (2002), the second stage degradation of pure quercetin occurs at 342°C to 428°C in the air atmosphere conditions. In addition, the second stage also involved the correspondence breakdown of hemicellulose and cellulose therein. Ram et al. (2014) reported that the second weight loss in *Carica papaya* leaf powder occurs between 200°C and 450°C and is linked to hemicellulose and cellulose breakdowns. Khelloufi et al. (2017) reported that the breakdown of cellulose and lignin in the shrub leaf was found sequentially at temperatures 384°C to 464°C and 464°C to 576°C. In DTA curves, the degradation of *faloak* leaf powder was found in the ranges of 259.80°C to 373.92°C identified with the exothermic peaks, with peak decomposition temperature found to be 364.67°C. Malucelli et al. (2018) also reported the similar reported that 2nd phase of degradation of *Dicksonia sellowiana* extract based on the DTA curve occurred at two prominent peaks, which are the endothermic peak found at

271.99°C and the exothermic peak found at 322.33°C, which is attributed to the degradation of non-oxidative-in-extract. In other studies, pure lignin's exothermic decomposition peak in the DTA curve was found at 340°C (El Moustaqim et al., 2018).

Table 1 The interpretation of the IR spectrum of *faloak* leaf powder

Wavenumber (cm^{-1})	Chemical Bond	The interpretation results
3305.92	O-H stretching	Phenols, Flavonoids, Tannin, Alkaloid, Saponin
3305.92	O-H water	Hydrate
2921.05	C-H aliphatic	Phenols
2854.27		
1727.64	C=O	Flavonoids, Tannin, Phenols, Saponin
1606.29		
1514.38	C-C aromatic	Phenols, Tannin
1439.70		
1372.92	O-H bending	Phenols, Flavonoids, Tannin
1313.32		
1246.54	C-O alcohol	Flavonoids, Phenols, Tannin, Alkaloids, Saponin
1246.54		
1153.20	C-O eter	Flavonoids, Tannin
1034.72		
1034.72	C-O-H	Tannin
1246.54		
1153.20	C-N stretching	Phenols, Alkaloids
1034.72		
882.49		
820.74	C-H aromatic	Flavonoids, Tannin, Phenols, Alkaloids
773.34		
509.82		
556.49	C-H aromatic	Flavonoids
773.34		
432.27	C-OH aromatic	Phenols

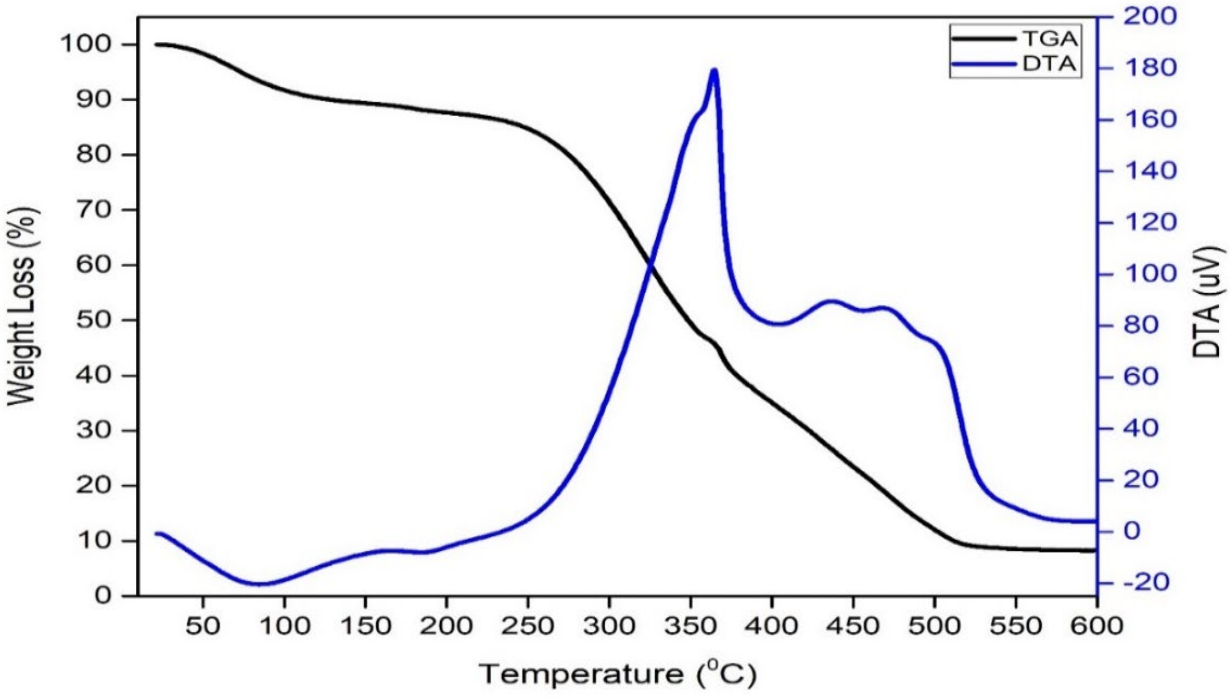

Figure 4 The TGA and DTA curve of *faloak* leaf powder

Based on TGA curves, the complex molecule broke down slowly and steadily throughout the third degradation stage in *faloak* leaf powder, which occurred at the range temperature of 521.92 to 600°C. Reza et al. (2020b) reported that the third stage of biomass decomposition was found at a temperature range of 441°C to 900°C. Ram et al. (2014) also reported that *Carica papaya* leaf powder degrades lignin at 450°C to 800°C. El-Sayed et al. (2023) also stated that hemicellulose breaks down between 180°C

and 340°C, cellulose breaks between 230°C and 450°C, and lignin thermally decomposes at temperatures over 500°C. Moreover, Da Costa et al. (2002) also stated that the third stage degradation of pure quercetin occurs at 428°C to 605°C in the air atmosphere conditions. Overall, thermal characterization results indicate that *faloak* leaf powder has relatively good heat stability until moderated temperature, so it has the potential to be applied in the production of food products that require moderate temperatures for processing and also can be applied in other related disciplines such as pharmaceuticals.

4 Conclusion

The phytochemical quantification and thermal characterization of *faloak* leaf powder were successfully investigated. The results revealed that the *faloak* leaf powder was found to have a high total phenolic content (89.26 mg GAE/g DW) and a high total flavonoid content (9.57 mg QE/g DW). *Faloak* leaf powder also has a particle size distribution ranging from 0.145 to 110.467 µm, with a median of 10.072 µm. According to the zeta potential test, the *faloak* leaf powder has a negative surface charge of -19.1 mV. The chemical bonds in FTIR spectra verified the presence of phenolic and flavonoid compounds in *faloak* leaf powder. The phenolic compounds were identified by their fingerprint chemical bonds, which included C-H aliphatic, C=O, C-C aromatic, O-H bending, C-O, C-N stretching, C-H aromatic, and C-OH aromatic bonds. In contrast, the chemical bond fingerprint of flavonoid compounds was the O-H stretching, C=O, O-H bending, C-O, C-O eter, and C-H aromatic bonds. Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) curves revealed that *faloak* leaf powder remained stable at moderate temperatures. As a result, *faloak* leaf powder shows the potential and promise to be employed as a functional ingredient in developing distinct functional food products or even applications in other related fields, such as pharmaceuticals.

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