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Influences of Different Drying Methods and Extraction Solvents on Total Phenolic and Flavonoids, and Antioxidant Capacity of *Moringa stenopetala* Leaves

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Abstract
M. stenopetala is a multipurpose plant having high nutritional and medicinal value. The aim of this study was to evaluate the effects of different drying methods and extraction solvents on the bioactive compounds in *M. stenopetala* leaves. Samples were dried using different drying methods and extracted using 50% and 70%ethanol, and 100%aqueous solvents. There was a significant difference ($P< 0.05$) among different drying methods and the extracting solvents. Freeze-dried samples gave the highest values of total phenolic contents (TPC) (57.2mgGAE/g dried powder) and total flavonoid contents (TFC) (130.30mgRE/g dried powder). The highest antioxidant activity was observed in freeze-dried 70% ethanol extract using DPPH and ABTS radical scavenging assays with the IC_{50} values of 32.43µg/mL and 13.11µg/mL, respectively. It was followed by room-dried 70% ethanol extracts. Therefore, 70% ethanol was found to be the best extracting solvent to get higher yield of TPC and TFC with antioxidant effect.

Keywords: Antioxidant compounds, Drying methods, Extraction solvents, *Moringa stenopetala*

Introduction
Moringa stenopetala has multipurpose uses such as source food, medicine, oil from seeds, water clarification and biofuel production [1]. Currently it has been observed that *M. stenopetala* leaves are on the market as powder form. It is commonly used as herbal tea, nutritional supplement and medicinal values. *Moringa olefiera* is the most extensively studied species while *M. stenopetala*, the most abundant species in Ethiopia, is now getting more attention for potential utilization.

As the nutritional value of Moringa varies due to different factors [2, 3], the yield and antioxidant activities of Moringa extracts varies based on the geographical location, type of varieties, stage of maturity and collection season [4-6]. Additionally, drying methods [7, 8], extraction methods, solvent type and ratios have shown significant effects on the bioactive content [9, 10]. As reported by [11] when cauliflower is dried at 40 °C in oven dryer, it rendered the highest yield of antioxidants whereas the air-dried at 25 °C gave the lowest yield. The storage and processing methods also have effects on the bioactive compounds [12, 13].

Drying is important to reduce the moisture content of fresh materials to extend the shelf life and reduce transportation cost. Different drying methods have showed significant influences on stability of bioactive compounds and their antioxidant capacity in addition to their effects on the physical and sensory quality [8, 14]. However, no reports were published on the effects of drying methods and extraction solvents on the bioactive components of *M. stenopetala* leaves. This study was therefore undertaken to determine the effects of different drying methods and extraction solvents of *M. stenopetala* leaves on total phenolic and flavonoid compounds and the antioxidant capacity of the extract.

Material and methods
Chemicals and reagents
All chemicals and reagents used were analytical grades. Aluminum chloride (Hopkin & William, England). Sodium hydroxide (Lobachem, India). Sodium carbonate (BDH, England). Potassium persulfate (Acros organics, USA). Sodium nitrite (Prolab, Paris) Folin-Ciocalteu's Phenol reagent, Rutin, Gallic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonicacid)) diammonium salt (Sigma-Aldrich, Germany).

Sample preparation

M. stenopetala leaves sample was collected from Arba Minch University's farm about 525km away from Addis Ababa, Ethiopia. The specimen was identified by the Botanist and deposited at the herbarium of Traditional and Modern Medicine Research Directorate, Ethiopia Public Health Institute with voucher number Debebe W.001.2016. The sample was washed using distilled water to remove the dirty matters. Then the sample was divided into six portions to dry using different drying methods (Fig. 1). Finally the samples were ground and allowed to pass through a sieve (20 mesh size) and kept in sealed plastic bag protected from light until extraction.

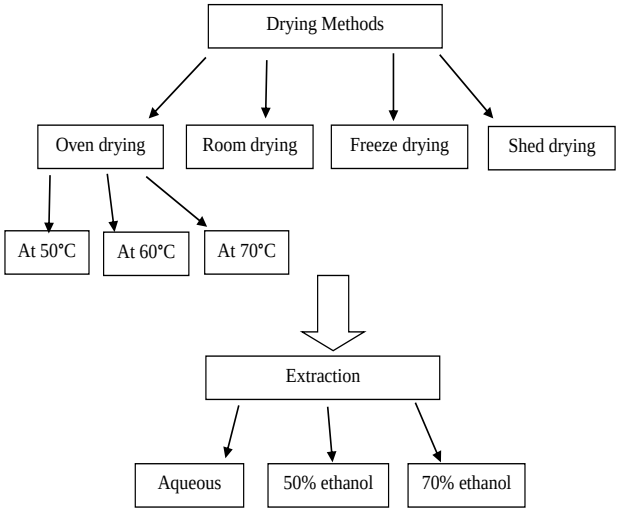

Fig 1: Shows different drying methods and solvent extraction of *M. stenopetala* leaves

Extraction of *M. stenopetala* leaves

Extraction was done according to Vongsak *et al.* [15] with some modification. Briefly, the samples were mixed with solvents (50%, 70% ethanol and aqueous) in the ratio of 1:40 (g/mL) in the flask. Then the flask was covered with aluminum foil to avoid light contact during extraction. Extraction was done with occasional shaking at 170 rpm using orbital shaker (VWR DS-500, USA) for 72 h, exhaustively. The extracts were filtered and concentrated using rotary evaporator (BÜCH Rotavapor R-200, Switzerland). Aqueous extracts were freeze dried (LABACON, Free zone 6, USA) and then kept at 3°C until analysis.

Moisture content determination

Moisture content of the fresh leaves sample and the dried samples were determined according to the method described by [16].

Determination of total phenolic contents (TPC)

The TPC of the extracts were done according Singleton and Rossi [17]. Gallic acid standard was used to develop standard curve. 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and mixed using a vortex mixer. After the mixture was kept for 8 min, 2 mL of 7.5% sodium carbonate was added and mixed again. The mixture was kept for 2 h in the dark at room temperature. The same procedure was used for the standard and blank. Then absorbance was measured at 765 nm using UV-Vis spectrophotometer (Shimadzu, UV-1800, Japan). The TPC in the extract was calculated from the standard curve and expressed as milligram of gallic acid equivalent per gram of leaves powder (mg-GAE/g dw) using the equation shown below.

$$TPC = c * v/m \quad \text{Eq. 1}$$

Where, TPC– total phenolic content (mg-GAE/g dw), c- concentration of gallic acid established from the calibration curve (mg/mL), v- volume of the extract (mL), m- dry weight of the leaves powder (g).

Determination of total flavonoids content (TFC)

The TFC was determined according to Adom and Liu [18] with slight modification. Rutin was used as a standard. 0.5 mL of the extract/ rutin was mixed with 0.15 mL of 5% (w/v) sodium nitrite and 2.5 mL of distilled water. The mixture was vortexed and left for 6 min at room temperature. 0.3 mL of 10% (w/v) aluminum chloride was then added to each sample, mixed and kept for more 6 min. This was followed by addition of 1 mL of 1.0 M sodium hydroxide subsequently 0.55 mL of distilled water. The mixture was mixed and kept for 15 min. Finally, the absorbance was measured at 510 nm. The TFC of the sample was expressed as mg rutin equivalent per g of leaves powder (mg-RE/g dw) as expressed above on the Eq. 1.

DPPH radical scavenging activity assay

The free radical scavenging activity of the extract was measured using the DPPH according to Brand-Williams *et al.* [19]. A 6 $\times 10^{-5}$ M DPPH solution was prepared in 80% methanol. 0.1 mL of the sample/standards of different concentrations using 80% methanol mixed with 3.9 mL of DPPH solution. The mixtures were then mixed and kept in the dark at room temperature for 30 min. The absorbance of the standards (ascorbic acid) and samples were measured at 515 nm using UV-Vis spectrophotometer. The percent of scavenging activity was calculated using the formula shown below. The half-maximal inhibitory concentration (IC_{50}) was calculated from the plotted graph and expressed as µg/mL.

$$\%\, Scavenging\ capacity = 1 - \left(\frac{Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ blank}\right) * 100 \quad \text{Eq. 2}$$

ABTS radical scavenging assay

The $ABTS^{\cdot+}$ radical scavenging assays were done according the method described by [20]. In Brief, ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting 7.0 mM concentration of ABTS solution with 2.45 mM potassium persulfate and incubated for 12 to 16 h in the dark at room temperature. This solution was then diluted using methanol to get an absorbance of 0.70 $\pm$ 0.02 at 734 nm using UV-Vis spectrophotometer. Then, 50 µl of sample extracts and standards of different concentrations were added to 5 ml of $ABTS^{\cdot+}$ solution and mixed thoroughly. The blank solution prepared using 80% methanol instead of the sample solution and assayed under the same conditions. The absorbance was then measured after 6 min at 734 nm using UV-Vis spectrophotometer. Then percent of radical scavenging activity of the extracts and the standards were calculated using the equation shown above on the Eq. 2. The IC_{50} value was calculated from the plotted graph and expressed as µg/mL.

Statistical analysis

The data were analyzed using JMP software Version 7.0.1 (SAS institute Inc., Cary, NC, USA). All experiments were

done in triplicate and expressed as mean ± standard deviation. The significance tests were performed by means comparisons using one-way analysis of variance (ANOVA) and student's t test. Differences were considered at a significance level of $P \leq 0.05$.

Results and Discussion

Effect of drying methods on residual moisture content dried sample

The effects of different drying methods and time on final moisture content of the dried sample are shown on Table 1. The samples were dried using different drying methods in time range from 10 to 72 h. The moisture content of the fresh sample was 74.62%. The final moisture content of the dried samples ranged from 5.67 to 8.17%. The final moisture contents of the dried samples were significantly different ($P<0.05$) among different drying methods.

Table 1: Effects of different drying methods on the moisture content of *M. stenopetala* leaves.

Drying methods	Drying time (h)	MC of fresh Sample (%)	Residual MC in dried samples (%)
Freeze drying	24	74.62 ± 0.02	6.67 ± 0.29^{c}
Room drying	72	74.62 ± 0.02	7.17 ± 0.29^{bc}
Shed drying	48	74.62 ± 0.02	7.67 ± 0.29^{ab}
Oven drying at 50 °C	21	74.62 ± 0.02	5.83 ± 0.29^{d}
Oven drying at 60 °C	16	74.62 ± 0.02	5.83 ± 0.29^{d}
Oven drying at 70 °C	10	74.62 ± 0.02	5.67 ± 0.29^{d}
CBDT	72	74.62 ± 0.02	8.17 ± 0.29^{a}

Means with different superscript letters in columns and rows ($^{a-d}$) indicate a significant difference ($P<0.05$) for different drying methods and extraction solvent ratios. CBDT- community based drying technique, MC- moisture content.

Samples dried in an oven at temperatures of 70°C, 60°C and 50°C took relatively shortest time of duration. On the other hand, samples dried at room temperature took longest time (72 h) compared to others. The variations of these drying time requirements may be attributed to the factors such as, relative humidity, temperature and airflow rate of the surrounding environment. In general, shorter drying time could be achieved with lower relative humidity and higher temperature. These conditions could be the possible contributing factors for the differences in the drying time and methods among the processed samples. Shed drying has the advantages of shorter duration compared to room drying which may be attributed to the lower relative humidity.

Effect of drying methods and extraction solvents on the extraction yield

There are different conventional and emerging technologies for extraction that can increase the extraction yield of a plant. Extract yield depends on the extraction methods, type of solvents and the mixture ratios when more than one solvents are employed during extraction. The yield of *M. stenopetala* leaves extract was ranged from 24.46 – 34.94% (Table 2). The highest extract yield was obtained for freeze-dried sample when extracted using 50% ethanol. In most cases, higher extract yield was obtained with 70% ethanol compared with 50% ethanol and aqueous extracts (Tables 2). This finding is in good agreement with the report of Do *et al.* [10]. The majority of plant constituents extract yield could be improved by the use of the combination of alcohol and water since both water and alcohol soluble compounds could solubilize all possible constituents [10].

Table 2: Effect of drying methods and extraction solvents on the extraction yield (%).

Drying methods	Extracting solvents		
	70% ethanol	50% ethanol	Aqueous
Freeze drying	33.72 ± 0.06^{cd}	34.94 ± 0.02^{a}	28.45 ± 0.07^{k}
Room drying	32.79 ± 0.07^{f}	29.55 ± 0.51 ^{j}	33.20 ± 0.21^{e}
Shed drying	34.18 ± 0.02^{b}	26.21 ± 0.20 ^{m}	32.42 ± 0.09^{f}
Oven drying at 50 °C	31.18 ± 0.15^{g}	30.28 ± 0.45^{hi}	27.50 ± 0.07^{l}
Oven drying at 60 °C	34.02 ± 0.14^{bc}	24.46 ± 0.12^{n}	27.80 ± 0.10^{l}
Oven drying at 70 °C	34.10 ± 0.16^{bc}	33.41 ± 0.20^{de}	30.03 ± 0.16^{i}
CBDT	33.43 ± 0.05 ^{de}	30.61 ± 0.44^{h}	30.64 ± 0.44^{h}

Means with different superscript letters in columns and rows ($^{a-m}$) indicate a significant difference ($P<0.05$) for different drying methods and extraction solvent ratios. CBDT- community based drying technique.

The yield of freeze-dried samples (34.94%) was significantly higher ($P<0.05$) than shed (34.18%) and oven dried samples (34.02%, 34.10%) at temperatures of 60°C and 70°C, respectively (Table 2). Lowest sample yield (24.46%) was observed when the oven-dried sample at a temperature of 60°C, which was extracted using 50% ethanol. This is by far better than the extracted yield of *M. stenopetala* leaves powder (17.3%) prepared in absolute methanol as reported by [5]. According to the report of [15] higher extract yield is obtained for dried *M. olifiera* leaves in aqueous decoction (59.24%) than 70% ethanol extract (40.5%). The extract yield increased for *L. aromatica* (33.67%) when using 50% acetone compared to other extraction solvents [10] and for *C. reticulate L.* higher yield (18.46%) when 80% ethanol was used for extraction [21]. The findings in the current study and the reports showed that the extracts yield of the plants depends on polarity and type of solvents Sultana *et al.* [22].

Total Phenolic Contents (TPC)

The TPC was affected by different drying methods and extraction solvents. This has an impact on the antioxidant activities and other bioactivity properties. It is important to select relatively the best drying method and extraction techniques that could preserve the phenolic constituents without degradation. The TPC of the freeze-dried sample extracted by 70% ethanol has 57.2 mg GAE/g dw which was significantly ($P<0.05$) higher than other drying (Table 3). This result is in agreement with the report of Vongsak *et al.* [15] in which 70% ethanol extract gave a yield of TPC (53.5 mg CAE/g of dw) from *M. olefiera* leaves. Aqueous extract prepared by oven drying at a temperature of 60°C had shown the next highest yield (36.67 mg GAE/g dw) of TPC (Table 3).

The aqueous extracts of freeze-dried samples has the lowest TPC (22.67 mg GAE/g dw) compared to the other drying methods and extraction solvents. On the contrary, according to the reports of Assefa *et al.* [23], the highest TPC (44.86 mg GAE/g) was obtained when *M. stenopetala* was prepared using aqueous decoction. This showed that even if the TPC of the sample are depend on drying methods, the extraction techniques employed may also determine to enhance the phenolic content of the extracts [21].

Table 3: Total phenolic contents of *M. stenopetala* extracts (mg GAE/g dw).

Drying methods	Extracting solvents		
	70% ethanol	50% ethanol	Aqueous
Freeze drying	57.2 ± 0.81^{a}	31.6 ± 0.40^{f}	22.67 ± 0.61^{n}
Room drying	31.87 ± 0.23^{f}	28.53 ± 0.61^{hi}	24.67 ± 0.61^{m}
Shade drying	32.8 ± 0.40^{e}	30.13 ± 0.46^{g}	24.67 ± 0.61^{m}
Oven drying at 50°C	33.6 ± 0.80^{cd}	30.13 ± 0.23^{g}	33.73 ± 0.23^{c}
Oven drying at 60°C	25.73 ± 0.23^{l}	27.87 ± 0.23^{ij}	36.67 ± 0.23^{b}
Oven drying at 70°C	26.8 ± 0.40^{k}	28.93 ± 0.46^{h}	27.07 ± 0.23^{k}
CBDT	32.93 ± 0.23^{de}	26.93 ± 0.23^{k}	27.47 ± 0.92^{jk}

Means with different superscript letters in columns and rows ($^{a-n}$) indicate a significant difference ($P< 0.05$) for different drying methods and extraction solvent ratios. GAE- Gallic acid equivalent, CBDT- community based drying technique, dw- dry weight of the leave powder

Therefore, drying methods, solvents type used for extraction and their ratios could affect the TPC [8]. Thermally drying methods such as sun, oven and microwave drying methods may also have a negative effect due to the reduction of the TPC of the leaves [7]. The difference in TPC of the extracts prepared from different drying methods and extraction techniques might be attributable due to oxidation and decomposition of bioactive compounds [8].

Total Flavonoid Content (TFC)

M. stenopetala leaves dried at different drying methods have shown a significant difference ($P<0.05$) on the TFC of the extracts. The TFC found to be highest for freeze-dried leaves extracted using 70% ethanol (130.53 mg RE/g dw). The observed result (Table 4) indicated that the TFC of *M. stenopetala* leaves highly dependent on the methods of drying and solvent type. According to the report of Vongsak *et al.* [15] the TFC of *M. olifiera* leaves extract using 70% ethanol found to be highest compared to other solvents. Although freeze-dried sample gave the highest values of TFC, the least TFC content was also obtained from aqueous extract (Table 4). Aqueous decoction of the leaves of *M. stenopetala* for 5 min yielded the highest total flavonoid (20.36 mg QE/g) when compared with 10 and 15 min decoction [23]. This indicates that even if the drying methods have significant impact on the total flavonoid of *M. stenopetala* extracts, the solvent type and ratios used for extraction may also have a significant effect ($P<0.05$). Therefore, selection of appropriate extracting solvent type and ratio are essential to maximize the yield of TFC of the plant extract thereby optimize nutraceutical products development.

Table 4: Total flavonoid contents of *M. stenopetala* extracts (mg RE/g dw)

Extracting solvents			
Drying methods	**70% ethanol**	**50% ethanol**	**Aqueous**
Freeze drying	130.53 ± 1.67^{a}	43.73 ± 0.46^{f}	14.00 ± 1.60^{m}
Room drying	68.00 ± 2.80^{b}	36.67 ± 0.61^{g}	13.47 ± 1.01^{m}
Shade drying	65.07 ± 3.00^{c}	41.6 ± 2.27^{f}	16.8 ± 1.60^{l}
Oven drying at 50°C	57.2 ± 0.60^{d}	33.87 ± 1.40^{h}	28.4 ± 1.44^{j}
Oven drying at 60°C	41.33 ± 2.44^{f}	31.2 ± 0.80^{i}	54.4 ± 0.40^{e}
Oven drying at 70°C	34.53 ± 2.20^{gh}	32.4 ± 0.40^{hi}	27.47 ± 0.83^{jk}
CBDT	53.6 ± 0.40^{e}	33.6 ± 0.40^{hi}	25.73 ± 1.85^{k}

Means with different superscript letters in columns and rows ($^{a-m}$) indicate a significant difference ($P< 0.05$) for different drying methods and extraction solvent ratios. RE- Rutin equivalent. CBDT- community based drying technique. dw- dry weight of the leave powder

Although oven-drying method showed relatively the fastest drying method, the TFC of the sample dried at 70 °C were very low. This might be attributed to the higher temperature that leads to the decomposition of the bioactive compounds as stated by Nguyen *et al.* [8]. Although higher drying temperature has advantage to remove moisture rapidly, the heat sensitive compounds will be decomposed. This result is agreed with the finding of the persimmon fruit dried using oven [24]. *P. amarus* leaves dried using microwave [8] and plant dried at far-infrared radiation at 65°C [25], reported that the TFC of the extract were very low. Therefore, drying temperature should considered minimizing the decomposition of the desired compounds.

DPPH radical assay

The DPPH radical scavenging activity of the 70% ethanol extracts in decreasing order were freeze-dried > room-dried > shed-dried >oven-dried at 50 °C > locally dried > oven-dried at 70°C > oven-dried at 60 °C (Table 5). This shows that antioxidant activity is also affected by drying conditions. This is in agreement with Anwar *et al.* [11] who reported that the highest antioxidant activity in Cauliflower dried at 40 ºC in oven-drier but the least anti-oxidant effect when it is air-dried at ambient temperature.

The antioxidant activity was also dependent extraction solvents. The freeze-dried sample extracted using 70% ethanol showed higher antioxidant activity (32.43 µg/mL) compared to 50% ethanol (58.75 µg/mL) and aqueous extracts (90.26 µg/mL) (Table 5). It was earlier reported that IC_{50} value for the radical scavenging activity of *M. stenopetala* leaves powder was 59.5 µg/mL when extracted using absolute methanol for two weeks period [5], and Assefa *et al.* [23] reported an IC_{50} value of 41.5 µg/mL for the aqueous decoction of *M. stenopetala* leaves powder. *M. olifiera* showed an IC_{50} values of 62.94 µg/mL when extracted with 70% ethanol,122 µg/mL when extracted with 50% methanol for three days and 78.15 µg/mL for aqueous decoction for 5 min [26]. These findings indicated that the extraction techniques and temperature have significant effects of the antioxidant effect of the extracts. According to this study, the best extracting solvent is 70% ethanol among other solvents used for extracting *M. stenopetala.*

Table 5: Effects of different drying methods and extracting solvents on DPPH radical scavenging activities of *M. stenopetala* leaves extracts (IC_{50} values in µg/mL).

Extracting solvents			
Drying methods	**70% Ethanol**	**50% Ethanol**	**Aqueous**
Freeze drying	32.43 ± 0.26^{a}	58.75 ± 1.25^{g}	90.26 ± 1.04^{p}
Room drying	35.09 ± 0.04^{b}	60.00 ± 0.35^{h}	81.55 ± 0.97^{n}
Shade drying	37.85 ± 0.13^{c}	59.62 ± 0.18^{gh}	84.95 ± 0.33^{o}
Oven drying at 50 ˚C	36.70 ± 0.06^{c}	39.91 ± 0.26^{d}	72.40 ± 1.36^{m}
Oven drying at 60 ˚C	65.04 ± 0.87^{j}	46.26 ± 1.20^{f}	40.04 ± 0.32^{d}
Oven drying at 70 ˚C	63.30 ± 0.48^{i}	43.38 ± 0.16^{e}	81.76 ± 1.44^{n}
CBDT	42.19 ± 0.13^{e}	66.93 ± 0.82^{k}	70.97 ± 0.94^{l}

Means with different superscript letters in columns and rows ($^{a-p}$) indicate a significant difference ($P< 0.05$) for different drying methods and extraction solvent ratios.IC_{50}- the half maximal inhibitory concentration. CBDT- community based drying technique.

Assessment of the extracting solvents cause variation of the IC_{50} values for different plants. For instance, *Kedrostis foetidissima* leaves extract in increased order of antioxidant effect reported for extracting solvents to be highest for methanol which is sequentially followed by chloroform, aqueous, acetone and petroleum ether, respectively [27]. In

addition, 80% methanol gave the highest antioxidant activity for Kinnow peel extract followed by 80% ethanol [21]. On the contrary, ethanol extracts had the highest antioxidant activity compared to methanol [10, 15].

ABTS radical assay

The constituents of plants are complex in nature. This may create difficulties to evaluate the antioxidant effects of the extract using single method. Therefore, ABTS an alternate approach for DPPH to evaluate the radical scavenging activity of the extract. It is found that the antioxidant assay responded by DPPH assay is varied from ABTS. This is illustrated by the report of Floegel *et al*. [28] who stated that higher antioxidant activity of strawberry when the plant extract was analyzed with DPPH assay than ABTS radical assay and vice-versa for blueberry extract. Therefore, $ABTS^{+\cdot}$ antioxidant evaluation technique was employed besides DPPH radical scavenging activity in the current study.

Table 6: Effect of different drying methods and extracting solvent on ABTS radical scavenging activities of *M. stenopetala* leaves extracts (IC_{50} value in µg/mL).

Extracting solvents			
Drying methods	**70% Ethanol**	**50% Ethanol**	**Aqueous**
Freeze drying	13.11 ± 0.05^{a}	28.57 ± 0.13^{h}	40.28 ± 0.29^{n}
Room drying	15.97 ± 0.12^{b}	28.23 ± 0.16^{h}	35.29 ± 0.31^{l}
Shade drying	17.16 ± 0.07^{c}	24.86 ± 0.16^{g}	38.23 ± 0.43^{m}
Oven drying at 50˚C	17.00 ± 0.11^{c}	17.80 ± 0.09^{d}	33.32 ± 0.42^{i}
Oven drying at 60˚C	28.32 ± 0.24^{h}	21.15 ± 0.10^{f}	20.88 ± 0.12^{f}
Oven drying at 70˚C	34.48 ± 0.07^{k}	19.58 ± 0.04^{e}	41.02 ± 0.77^{o}
CBDT	17.20 ± 0.05^{c}	35.22 ± 0.02^{l}	33.90 ± 0.45^{j}

Means with different superscript letters in columns and rows ($^{a-n}$) indicate a significant difference ($P < 0.05$) for different drying methods and extraction solvent ratios.IC_{50}- the half maximal inhibitory concentration. CBDT-community based drying technique.

In this study, ABTS radical scavenging activity of the extracts found to be significantly ($P < 0.05$) different among the drying methods and extracts (Table 6). Among the 70% ethanol extracts, the freeze-dried samples showed the lowest IC_{50} values, which was followed by room-dried, shed, oven-dried at 50 °C (Table 6). Concerning solvent ratios, 50% ethanol extracts showed higher antioxidant activity for oven-dried sample at a temperature of 50°C followed by the dried sample at 70 °C. This showed that oven-dried samples could have better antioxidant activity when using 50% ethanol. When the aqueous extracts were concerned, oven-dried at 60 °C had better antioxidant activity followed by oven-dried at 50 °C. The aqueous extract has lower antioxidant effect compared to other drying methods except oven-dried sample at a temperature of 60 °C. The freeze-dried aqueous extract is the one that showed lowest radical scavenging effect (40.28 µg/mL).

The IC_{50} values of ABTS radical scavenging assay was lower when it was compared with the IC_{50} values of DPPH radical scavenging activity. This might be due to the presence of various types of compounds with antioxidant effects that can be scavenged by ABTS rather than DPPH. [28] reported that ABTS assay is better than DPPH assay for the pigmented and hydrophilic extracts. ABTS assay showed significantly higher antioxidant activities for fruits, vegetables and beverages [28].

Conclusion

M. stenopetala leaves possessing higher bioactive compounds yield and antioxidant activity when the samples were dried by freeze dryer. The total phenolic and flavonoid content was lower in oven-dried leaves extracts of *M. stenopetala* leaves. This might be due to thermal destruction of bioactive compounds. In general, 70% ethanol rendered the highest yield of total phenolic and flavonoids contents compared to the aqueous and 50% ethanol extracts. Therefore, 70% ethanol is the best extraction solvent for extraction of TPC and TFC from *M. stenopetala* leaves with high antioxidant effects. *M. stenopetala* leaves extract showed the higher antioxidant activity to be used as a potential source of antioxidants for nutraceutical and pharmaceutical industries.

Conflicts of interest

All authors declare no conflicts of interest.

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