Egypt. J. Chem. **Vol. 64,** No. 12 pp. 7583 - 7589 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Effect of Various Extraction Methods and Solvent Types On Yield, Phenolic and Flavonoid Content and Antioxidant Activity of *Spathodea nilotica* Leaves

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Abstract

Phytochemicals are gaining interest as a new source of natural pharmaceuticals to replace synthetic ones, which are controlled owing to potential health hazards and toxicity. A comparison of extract yield, phenolic, flavonoid content and antioxidant activity in various extraction methods were studied. Extraction with different solvent polarity including chloroform (CF), ethyl acetate (EtOAc), acetone (AC), methanol (MeOH), and distilled water (DW) was prepared using maceration extraction (ME), one of the traditional methods and two methods of recent extraction techniques also known as "Green Extraction" techniques, ultrasound-assisted solvent extraction (UASE) and microwave-assisted solvent extraction (MASE). The antioxidant activity of the extracts was measured using the DPPH method of the antioxidant assay. Higher phenolic (194.3±1.5 and 191.7±0.4, respectively) were found in the methanolic leaf extracts in the case of MASE and UASE than maceration extraction (ME). Meanwhile, DW extract showed the highest flavonoids (174.3±1.0 and 167.4±1.0, respectively) contents by using UASE and MASE followed by methanolic extract (140.1±0.6 and 136.6±1.1, respectively) compared with the conventional extraction technique. The extraction techniques, as well as the solvent polarity and time of extraction, influenced extract yield, total phenolic and flavonoid content, and antioxidant activity.

*Keywords:*Ultrasonic-assisted extraction; Maceration; Microwave-assisted extraction; Antioxidant activity; solvent effects.

1. Introduction

Plants are a rich source of bioactive substances that can be utilized to produce novel medications. Bioactive compounds that have been isolated are used as both precursor materials for drug synthesis in the lab and as a model for producing biologically active molecules. Additionally, basic plant materials must undergo phytochemical processing to optimize the concentration of recognized components while retaining their activity [**1**].

Extraction is a critical step in the phytochemical processing pathway for finding bioactive compounds in plant materials. Also, factors enhancing the extraction process such as temperature, duration, properties of solvents, the particle size of plant materials and cost have to be considered to optimize the extraction process and increase the extraction efficiency [**2, 3**]. A good extraction method is essential for standardizing herbal products since it removes soluble components that are needed while leaving others that are not. There is evidence that the solvents employed in the extraction process have an effect on the kind and amount of secondary metabolites recovered from the plants. It is, therefore, crucial to select an appropriate extraction solvent as well as extraction technique in order to obtain the required biological activity from these extracts. Furthermore, for purposes of upscaling, such as from bench stage to pilot plant level, selecting the proper extraction method and optimizing various parameters are crucial. Traditional extraction processes include maceration, percolation, infusion, decoction, and hot continuous extraction are among the most often utilized [**4**]. In recent years, alternative approaches such as supercritical fluid extraction (SFE), microwave assisted solvent extraction (MASE), and ultrasound-assisted solvent extraction (UASE), have gotten a lot of attention [**5, 6**].

As a result of its speed and ability to increase recovery yields of the targeted metabolites compared to traditional techniques, the usage of green extraction techniques includes SFE, MASE, and UASE for extracting phytochemicals from plants has been gradually rising. These methods are also

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Receive Date: 17 September 2021, Revise Date: 23 September 2021, Accept Date: 30 September 2021
DOI: 10.21608/EJCHEM.2021.96557.4518

environmentally friendly in terms of solvents and energy use. Also, the yield is similar to that of traditional extraction, and in certain conditions, it is considerably much higher [**6-12**]. However, multiple studies have found that the yield of extracts, as well as the bioactivities of extracts generated using various extraction methods, vary [**13**]. Therefore, the purpose of the current study was to investigate the effect of five solvents (chloroform, ethyl acetate, acetone, methanol and water), with various extraction techniques on phytochemical contents, the yields of extraction and antioxidant activity by DPPH radical scavenging of *Spathodea nilotica* leaf extract.

2. Experimental

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1- picrylhydrazyl (DPPH), aluminium chloride, sodium carbonate, chloroform, ethyl acetate, acetone, methanol, gallic acid, rutin, and ascorbic acid were purchased from Sigma-Aldrich (USA and Fluka, Switzerland). All the other chemicals were of analytical grade.

2.2. Plant material

Fresh leaves of *Spathodea nilotica* were collected from Orman botanical garden, Giza, Egypt, in September 2020. The leaves were kindly authenticated by a botanist at the Horticulture Research Institute, Giza, Egypt. The leaves were air-dried at room temperature in the shade, and then ground into a powder with an electrical blender through a 24 mesh sieve, and were kept in airtight bottles at room temperature (28±2ºC) in the dark until used.

2.3. Extract preparation

2.3.1. Maceration extraction (ME)

About 5 g of air-dried powdered leaves of *S. nilotica* were macerated with 50 ml each of CE, EtOAc, AC, MeOH, and DW separately in a conical flask and the flasks were placed on a shaker (Labconco, USA) under constant stirring at 150±2 rpm with controlled temperature (28± 2°C). Plant materials were extracted for three successive days at room temperature with constant shaking. The combined extract was evaporated to dryness under reduced pressure at 45ºC using a rotary evaporator (R300, BUCHI, Switzerland) to remove the CE, EtOAc, AC, MeOH, and DW after being filtered through a Whatman filter paper No. 1.

2.3.2. Ultrasound assisted solvent extraction (UASE)

About 5 g of air-dried powdered leaves of *S. nilotica* with 50 ml each of CE, EtOAc, AC, MeOH, and DW separately in conical flasks. The extraction process was done by placing the conical flasks in a Probe Sonicator homogenizer (Benchmark Scientific, USA, 150 W, 25 kHz) for 5, 10 and 20 min. The extracts were similarly treated as described in traditional extraction to obtain dried UASE extract of *Spathodea nilotica*.

2.3.3. Microwave assisted solvent extraction (MASE)

About 5 g of air-dried powdered leaves of *S. nilotica* with 50 ml of each CE, EtOAc, AC, MeOH, and DW in Pyrex conical flasks. Extraction was carried out by putting the flasks on a rotating surface and exposing them to microwave irradiation (LG Electronics, China, Model MS-3043bars/00, 1250 W, 2500 MHZ) for four cycles (30 sec. each) with a cooling step in between. The beakers were left for temperature stabilization once the heating process was completed (1 min). As previously mentioned, the extracts were centrifuged and concentrated. At 4 °C, samples of dried extract were stored in an airtight container.

2.4. Determination of total phenolic content (TPC)

The Folin-Ciocalteu reagent was used to determine the amount of total phenolic in *S. nilotica* plant extract. The results were expressed as mg gallic acid equivalents (GAE) mg GAE/g plant extract [**14**].

2.5. Determination of total flavonoid content (TFC)

The total flavonoid content (TFC) in the plant extract of *S. nilotica* was determined using the $AlCl_3$ method described by Lamaison and Carnet [**15**]. The results were calculated as mg rutin equivalent (RE)/g plant extract.

2.6. Antioxidant activity

2.6. DPPH radical scavenging activity

All extracts from *S. nilotica* leaves were tested for DPPH radical-scavenging activity using a modified approach developed by Brand-Williams *et al.*, [**16**].

Statistical analysis

All data was expressed as mean ± SD for three replications. Analysis of variance was used to do statistical analysis of the data and the Duncan test was used to assess for significance, which permitted multiple comparisons among the data to individualize the significant differences. If $p < 0.05$, differences were considered significant. SPSS version 19.0 was used to do the statistical analysis.

3. Results and discussion

3.1. Extraction and extraction yield

Plants often have low concentrations of biologically active compounds. An extraction technique can produce high-yield extracts with minimum changes to the extract's functional characteristics [**17**]. Several studies have found that extracts obtained using different extraction methods have varying biological activity. Therefore, the suitable extraction technique and solvent must be established based on the sample matrix quality, analyte chemical characteristics, efficiency, matrix analyte interaction, and required properties [**18, 19**]. Heat is transported from the surface by convection and conduction in traditional extraction; however, solvent extractability is mainly depending on the compound's solubility in the solvent, the product's mass transfer kinetics and the strength of the interaction between the solute and the matrix, all of which have corresponding limitations on heat and mass diffusion rate.

UASE is a method of extracting specific targeted molecules from diverse matrices using solvents and sound waves of high intensity and frequency. Plant cell walls are disrupted by the propagation and interaction of sound waves, which alters the physical and chemical properties of materials exposed to ultrasound. Hence, extractable compounds are released more easily, and mass transfer of solvent from the continuous phase into plant cells is improved. In MASE, microwave energy and solvents are utilized to extract targeted compounds from plant materials. Targeted molecules can migrate more quickly from matrices to their surroundings when temperature and pressure are highly localized. When compared to traditional extraction method, UASE and MASE recoveries are equivalent or better. On the other hand, UASE and MASE, offer the greatest advantages in terms of extraction time and solvent consumption. As a result of this work, we were able to determine how different extraction technique influenced the yield and phytochemical characteristics of *S. nilotica*.

The extract yield of *S. nilotica* leaves obtained by ME, UASE and MASE techniques using CE, EtOAc, AC, MeOH, and DW separately is illustrated in **Table 1**. As a measure of the effect of the extraction conditions, the extraction yield (weight of extract/weight of dried plant materials) was utilized. In ME, MeOH extract yield (13.8%) was maximum followed by DW and CE respectively. For UASE three time periods were used 5, 10 and 20 minutes. The extraction yield increased as the exposure period increasing to ultrasonic wave intensity.

It is obvious from the results in **Table (1)**, that extending the exposure period from five to twenty minutes increased the extraction yields of crude *S. nilotica* leaves. Compared to maceration (ME), UASE also decreased the extraction time at room temperature and extraction yield. On the other hand, UASE (for 20 min. exposure) and MASE were found to be quite similar. The extract yield of MASE was increased within 4 cycles (30 sec.) exposure to microwave radiation. UASE and MASE had the greatest extract yields, whereas ME had the lowest.

The maximum yield in MASE could be due to direct heat generation within the volume, which affects heating kinetics significantly, as well as the effects of pressure on the structure of the cell wall membrane, resulting in a higher and faster rate of solute diffusion or partition into the solvent from the solid matrix [**20, 21**]. A high-intensity ultrasound sonication effect may be responsible for the highest extraction yield in UASE, ultrasound in the solvent produces cavitation, which facilitates the dissolution and diffusion of the solute as well as heat transfer, which improves the extraction efficiency. Microwaves generate heat by interacting with water and other polar substances in plant matrix through ionic conduction and dipole rotation processes. By transferring energy and mass simultaneously, a synergistic effect is created to speed extraction and enhance yield. [**22**].

3.2. Total phenolic and flavonoid contents

The present was designed to compare the effective extraction techniques and solvents type on yield, phenolic and flavonoid content, and their corresponding antioxidant activity. For this purpose, 25 different extracts were investigated.

Phenolic and flavonoid content varied in different types of extracts of *S. nilotica* leaves and the results are summarized in **(Table 1)**. As shown in **(Table 1)** the highest amount of phenolic content (194.3±1.5 mg GAE/g extract) was obtained by using MASE technique with methanol as a solvent. The lowest phenolic content (32.15± 0.3 mg GAE/g extract) was observed using UASE for five minutes of exposure with CF as a solvent. The TPC of the extract obtained by using maceration ranged from 33.18±0.6 to 170.6±0.6 mg GAE/g extract. MeOH, DW, EtOAc and AC extracts showed the highest TPC of 170.6±0.6, 161.3±1.4, 118.0±1.3 and 76.92±0.1 mg GAE/g extract, respectively, while CF extract was the lowest one in TPC (33.18±0.6 mg GAE/g extract).

Total flavonoids contents (TFC) revealed the best extraction technique is UASE for 20 min exposure with DW extract, content of flavonoid is (174.3±1.0 mg RE/g extract). The lowest content of flavonoid (25.66±0.1 mg RE/g extract) was determined when using UASE for five minutes' exposure with CF as a solvent. The TFC by using maceration varied from 25.90 ±0.2 to 153.0±1.0 mg RE/g extract. The results showed that DW, MeOH, EtOAc and AC extracts had

the highest total flavonoids content, while the lowest TFC was observed by CF extract.

For USAE (for 20 min. exposure) and MASE (4 cycles; 30 sec.), there was no significant difference in the TPC and TFC in all extracts. It can be concluded based on the results in (**Table 1**) that USAE and MASE better extraction methods and significantly affected the antioxidant activity and TPC and TFC than traditional extraction (i.e. ME).

3.3. Antioxidant activity

3.3.1. DPPH free radical scavenging activity (DPPH FRSA)

The antioxidant activity of the various extracts prepared using ME, UASE, and MASE techniques was determined using the DPPH assay. The results were expressed in terms of DPPH inhibition as a percentage i.e. percentage of antiradical activity (**Table 1 and Fig. 1**). As shown in **Table (1)**, there was noticeable variability in the antioxidant activity of the tested plant extracts in the case of UASE, and MASE than ME technique.

Antioxidant of the extracts obtained by using ME varied in the following order: MeOH > AC > DW > EtOAc > CF. Furthermore, antiradical activity in the case of USAE for 20 min. and MASE for 4 cycles (30 sec. each) showed the highest DPPH scavenging activity of the all tested plant extracts except chloroform extract which had the lowest DPPH scavenging activity value in both UASE, and MASE techniques.

Table 1. Antioxidant activity (DPPH inhibition percentage), TPs[p], TFs[f] and yield in various extract polarities from the dry powdered leaves of *S. nilotica* prepared by using different extraction methods.

Extraction Method	Extract	Yield (%)	% Antioxidant activity (50µg.ml^{-1})	**TPs** (mg GAE/g extract)	**TFs** (mg RE/g extract)
ME	CF[a]	12.1±0.0^{g}	4.91±0.1^{o}	33.18±0.6^{n}	25.90 ±0.2^{q}
	EtOAc[b]	11.6±0.1^{h}	53.4±1.5^{jk}	118.0±1.3^{h}	107.6±0.9^{j}
	AC[c]	10.8±0.3^{hi}	82.2±0.4^{cde}	76.92±0.1^{l}	69.13±0.0^{lm}
	MeOH[d]	13.8±0.2^{cd}	92.0±1.4^{ab}	170.6±0.6^{e}	107.6±1.5^{j}
	DW[e]	12.2±0.5^{g}	61.0±1.7^{hi}	161.3±1.4^{f}	153.0±1.0^{d}
UASE	CF[a*]	11.2±0.2^{h}	4.45±0.3	32.15±0.3^{o}	25.66±0.1^{q}
	CF[a**]	11.8±0.3^{gh}	5.38±0.2^{mn}	33.87±0.4^{n}	27.82±0.1^{p}
	CF[a***]	12.2±0.3^{g}	5.93±0.1^{m}	36.16±0.5^{m}	30.03±0.4^{o}
	EtOAc[b*]	11.0±0.2^{h}	48.2±0.8^{l}	83.14±1.5^{kl}	68.64±1.3^{mn}
	EtOAc[b**]	11.6±0.4^{h}	58.9±0.7^{j}	102.4±1.4^{hi}	90.74±1.1^{k}
	EtOAc[b***]	12.4±0.3^{g}	70.5±0.6^{e}	129.2±1.0^{g}	117.1±1.2^{g}
	AC[c*]	9.00±0.2	78.5±0.9^{d}	85.40±0.4^{k}	67.84±0.2^{n}
	AC[c**]	13.0±0.1^{ef}	85.2±1.3^{cd}	86.51±0.6^{jk}	69.05±0.1^{lm}
	AC[c***]	13.2±0.3^{e}	87.2±0.5^{c}	87.62±0.9^{j}	71.23±0.2^{l}
	MeOH[d*]	14.0±0.4^{d}	93.0±0.6^{ab}	187.3±1.4^{bc}	109.3±1.3^{i}
	MeOH[d**]	14.8±0.2^{c}	94.0±0.5^{a}	189.5±0.9^{b}	129.4±1.5^{f}
	MeOH[d***]	16.2±0.3^{b}	95.0±0.3^{a}	191.7±0.4^{ab}	140.1±0.6^{d}
	DW[e*]	14.2±0.6^{d}	65.0±0.9f^{g}	181.7±1.4^{d}	162.6±1.6^{cd}
	DW[e**]	14.5±0.5^{cd}	67.0±0.7^{f}	185.1±1.8^{c}	170.4±1.4^{ab}
	DW[e***]	18.2±0.4^{ab}	70.0±0.4^{e}	189.5±1.2^{b}	174.3±1.0^{a}
MASE	CF[a]	12.8±0.4^{g}	5.81±0.3^{m}	35.15±0.6^{mn}	28.97±0.2^{op}
	EtOAc[b]	13.0±0.3^{ef}	66.1±0.2^{fg}	128.1±0.5^{gh}	115.2±1.0^{gh}
	AC[c]	14.0±0.7^{c}	86.2±1.0^{c}	88.38±0.9^{j}	71.15±0.5^{l}
	MeOH[d]	16.6±0.5^{b}	94.0±0.4^{a}	194.3±1.5^{a}	136.6±1.1^{e}
	DW[e]	19.6±0.6^{a}	63.0±0.6^{h}	185.8±2.0^{c}	167.4±1.0^{bc}

[a]CF: Chloroform; [b]EtOAc: Ethyl acetate; [c]AC: Acetone; [d]MeOH: Methanol; [e]DW: Distilled Water; * 5 min.; ** 10 min.; *** 20 min. Values are expressed as mean ± S.D. (n=3). Means within each column with different letters (a-q) differ significantly ($P < 0.05$) between MASE, UASE, and ME. TPs: Total phenolics, TFs: Total flavonoids.

It is noteworthy that the antioxidant ability of the plant extracts basically depends on the composition of the extracts, the hydrophobic or hydrophilic nature of the antioxidants, type of solvent and method used for the extraction process [**23**]. Thus, this wide range of antioxidant activity may be attributable to the wide variety of antioxidant components present in plants [**24**], and its dependence on the type of solvent and method used for extraction [**23, 25**].

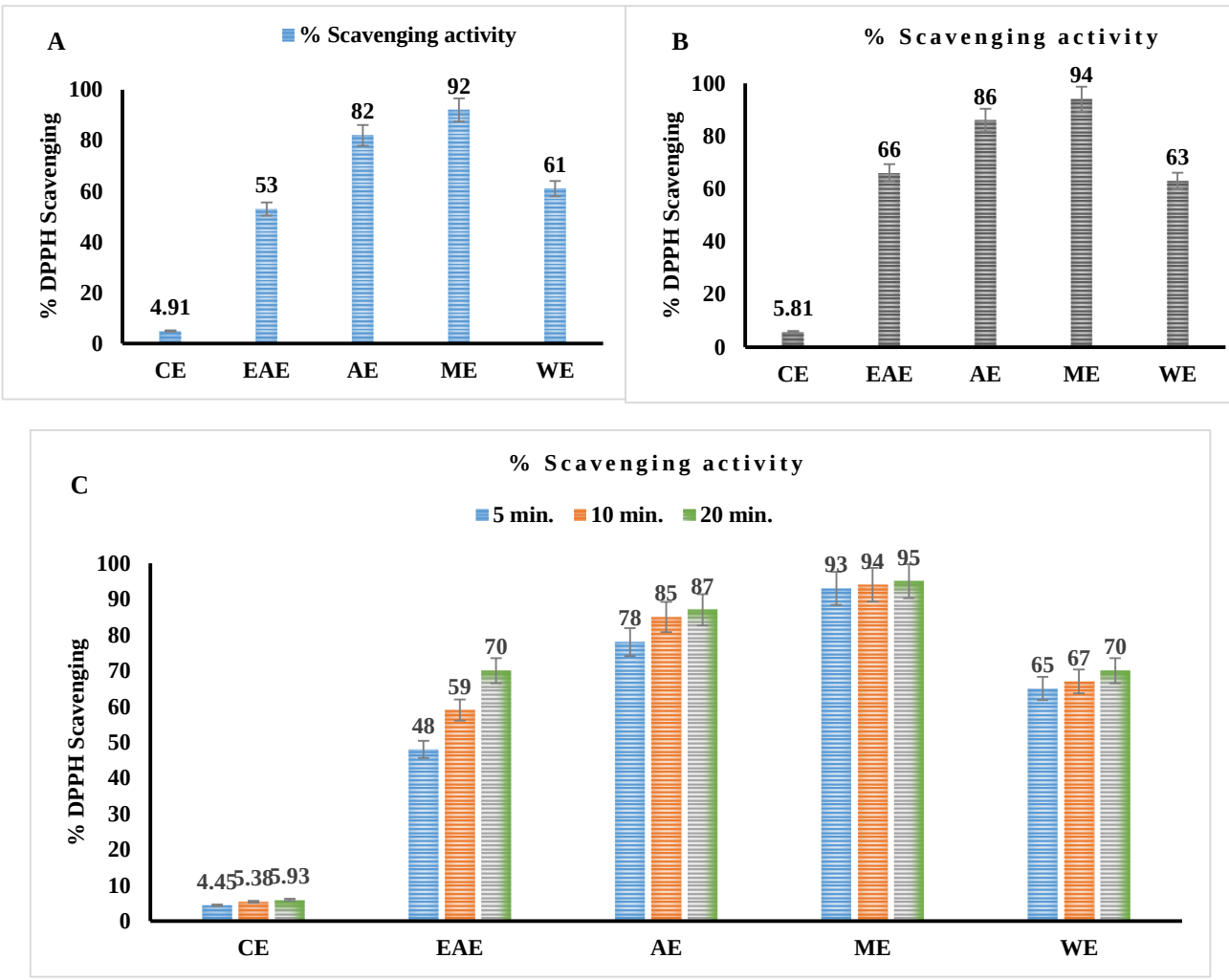

Fig.1. Effec of various solvent used and time period with respect to extraction technique on antioxidant activity evaluated by the DPPH radical scavenging assay in leaves of *Spathodea nilotica*; A: ME; B: MASE; C: UASE.

Considering the impact of solvent type, according to this study MeOH extracts obtained by UASE and MASE techniques has the best antioxidant activity (95.0±0.3 and 94.0±0.4) respectively. The highest antioxidant activity was observed in the MeOH extract obtained by UASE in twenty minute's exposure (95.0±0.3), followed MeOH extract produced by MASE (94.0±0.4), and then MeOH extract obtained by maceration (92.0±1.4). Finally, MeOH extracts obtained by various extraction methods had the greatest levels of phenolic content and antioxidant activity.

Moreover, Tiwari and Tripathi, **[26]** suggested that leaf extracts may contain various antioxidant components with different polarities. Therefore, the results obtained in this study depend on the type of antioxidant components isolated by each of the solvents and methods used.

3.3.2. Correlation between the TPC and TFC, and antioxidant activity

The correlation (R^2) between the antioxidant capacity and both the total phenolics and the total flavonoids contents is shown in **Fig. 2**. The correlation between the antioxidant activity and the phenolics content was 0.89 (**Fig. 2A**) whereas with total flavonoids content had " R^2" of 0.55 (**Fig. 2B**).

These results indicated that phenolic and flavonoid compounds contributed partially of 89.0% and 55.0%, respectively, to DPPH FRSA in the plant extracts studied. It should also be noted that the scavenging effect is not restricted to phenolic and flavonoid components, but also includes the presence of other antioxidant metabolites in the extracts that contribute to the activity directly or indirectly.

These data are in accordance with Tawaha et al., **[27]** and Borneo et al., **[28]** who indicated a linear correlation of TPC and TFC with antioxidant activity.

Eluent: *n*-hexane/EtOAc (80/20, v/v). Product **5g** was separated as colorless crystals, yield 68 %. mp

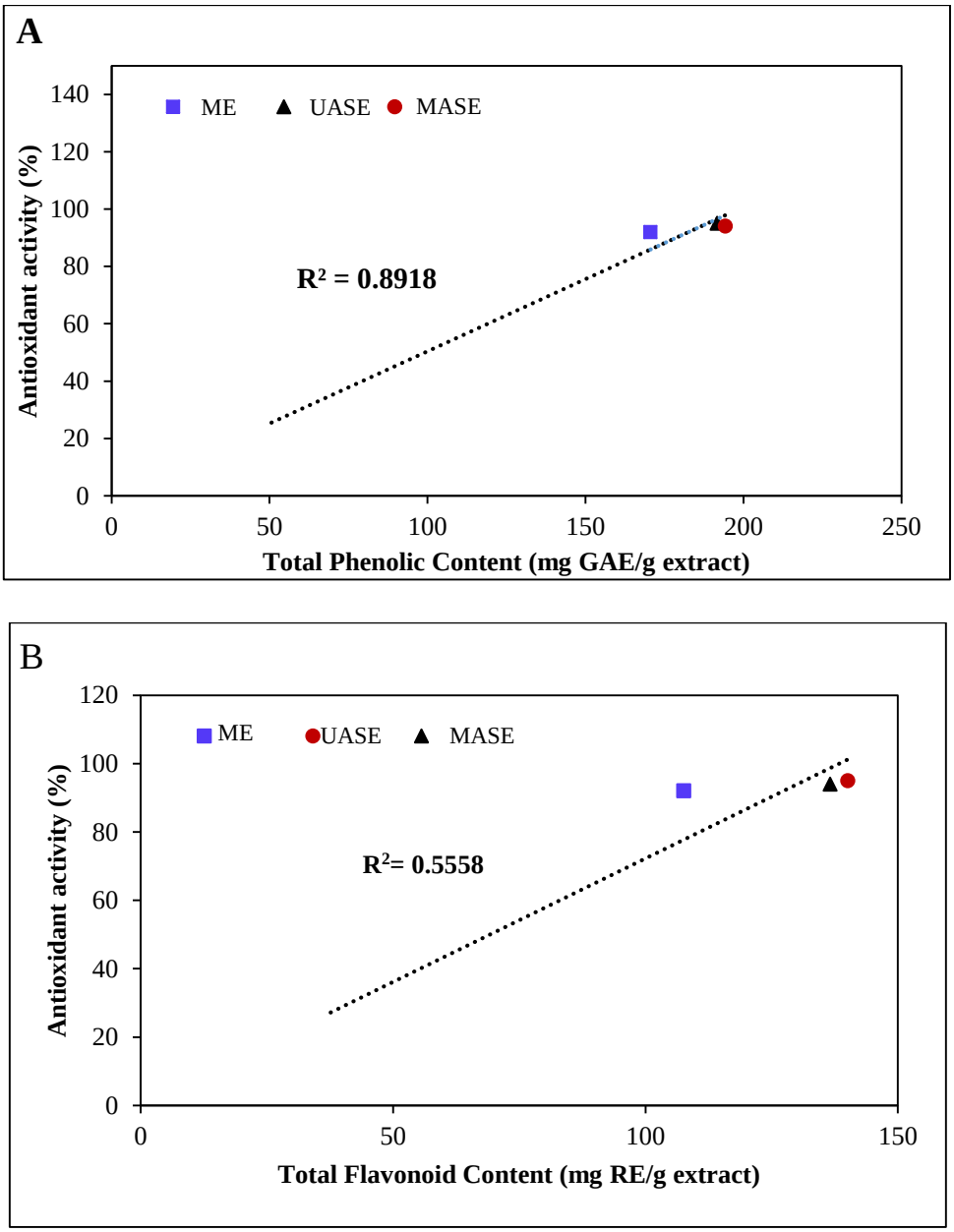

Fig. 2. Correlation between antioxidant activity (%) and TPC (A) and with TFC (B).

4. Conclusion

Finally, the findings of this investigation revealed that the nature of solvents and extraction techniques affect the yield, total phenolic and total flavonoid contents and their antioxidant properties in various degrees. Also, the results showed that the extracts of *Spathodea nilotica* contained significant quantities of phenolic and flavonoid compounds and had strong antioxidant activity, suggesting that they may be regarded prospective sources of potent antioxidants. Furthermore, antioxidant activity was shown to be significantly related to phenolic and flavonoid levels. Higher antioxidant activities values were found in methanol extracts in the case of UASE and MASE with larger levels of phenolic and flavonoid content, while the lowest phenolic and flavonoid content were observed by chloroform extracts with the lowest antioxidants levels.

5. Conflicts of interest

The authors declare no conflict of interest, or otherwise.

6. Acknowledgments

The authors gratefully acknowledge to Fayoum University, Egypt for the support of this work.

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