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Total Phenolic, Total Flavonoids Content and Antioxidant Activity of *Mangifera* sp. Leaf Extracts

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ABSTRACT

Mangifera sp. is a versatile plant that was reported to have various bioactivities, however only the fruits have gain popularity due to it sweet flesh and been known worldwide. It has a potential source of flavonoids and carotenoids, which makes them a nutritious functional food to consume. This study focused on determination of the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of the *Mangifera* sp. leaves extract in different water extraction methods. The TPC and TFC was determined by using Folin-Ciocalteu method and aluminium chloride method respectively while antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and ferric reducing antioxidant power (FRAP) assay. Soxhlet extraction (SXE) produced the highest extraction yield compared to microwave extraction (MWE). MWE at 8 minutes extract showed the highest TPC (262.13±0.05 mg GAE/g). SXE showed the highest TFC (413.46±0.77 mg rutin/g). The highest antioxidant activity was recorded in MWE 8 minutes extract through DPPH and FRAP assays compared to other two MWE extract and SXE extract.

Keywords: Mangifera sp., extraction method, phenolic, flavonoid, antioxidant

INTRODUCTION

Mangifera sp. is a popular evergreen fruit tree natural native to South-Eastern Asia. It is originally came from tropical Asia, the largest number of this species present mostly in Borneo, Java, Sumatra and Malaysia (Salahuddin et al., 2016). *Mangifera* contains approximately 69 different species and *Mangifera indica* is the typical species from the same genus (Taghvaei & Jafari, 2015). The leaves are various in shapes for instance oblong, linear- oblong, oval-lanceolate, ovate, obovate-lanceolate and round-oblong (Parvez, 2016). *Mangifera* sp. fruit, mango is an incredibly rich source of polyphenols, a large group of natural micronutrients found in plants with health advantages. A part from that, all different parts of this plant are utilized for their medicinal properties (Lauricella et al., 2017). The extracts from its leaves and tree bark have been useful for anti-malarial, antimicrobial, anti-inflammatory and antifungal (Kingne et al., 2018).

Antioxidants are compounds that block or diminish oxidative ions by free radicals into target particle (Oroian & Escriche, 2015). The oxidation processes are developed because of atmospheric oxygen or relative oxygen species (ROS), which is highly unstable and able to oxidize atoms or organic molecules such as proteins, lipids, and nucleic acids. During oxidation reactions, free radicals convey free unpaired electrons which cause them excessively unstable and able extracting an electron from different particles to accomplish stability causing them accumulated and collapsing cellular function resulting in generation of pathological states such as aging, cancer, stroke and diabetes.

The leaves extract of *Mangifera* sp. shows possible use in pharmaceutical applications as a chemo-precaution agent for illness that relates to oxidative pressure. Previous study by Laulloo et al. (2018) using a sample from Mauritius have reported a high antioxidant activity from the EtOAc leaves extract correlated to the high content of its TPC and TFC. A part from that, they also managed to identify many secondary metabolites from the leaves of the *Mangifera indica* and concluded that the presence of these metabolites is responsible for good antioxidant and antibacterial properties which can be exploited for its potential use as a supplementary fodder for ruminants. However, only a few studies recorded the bioactivities of the *Mangifera* sp. leaves originated from Asia.

Hence, there is a need to discover the potential natural antioxidants that capable to scavenge radicals to protect living organisms from disease and prevent oxidative rancidity of lipids. Therefore, this study is carried out to determine the antioxidant activities of *Mangifera* sp. leaves water extracts from two different extraction techniques.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, ethanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, potassium acetate, aluminium chloride, rutin, 2,2-Diphenyl-2-hydrazyl (DPPH), ascorbic acid, sodium acetate, glacial acetic acid, 2,3,5-triphenyltetrazolium chloride (TPTZ), hydrochloric acid and ferric chloride.

Plant materials

The *Mangifera* sp. leaves were collected from Kampung Jati, Gong Badak (5.3901^{0}N, 103.0836^{0}E) and in front of Faculty Informatics and Computing, Universiti Sultan Zainal Abidin, Besut Campus, Terengganu. Fresh and healthy leaves were chosen and the fresh weight was recorded. Leaves were washed under running tap water to clean it from dirt and left to dry at room temperature. Then, the leaves were placed in drying oven at 30^{0}C for three days. The dried sample was weighed and crushed.

Leaf extract preparation

Water was used as an extraction solvent for both extraction methods.

Soxhlet extraction (SXE)

Extraction was carried out by using a conventional Soxhlet apparatus which consist of distillation flask, thimble holder and a condenser. 7 g of the crushed *Mangifera* sp. leaves were placed in the extraction chamber, whereas 200 mL of distilled water was placed in the round bottom flask. The leaves were extracted for about 3 hours in a Soxhlet apparatus. When completed, the extraction water was collected. The extracts were concentrated using a rotary evaporator and then kept in -80^{0}C. After that, the extracts were freeze dried until it became a powder form. The extracts then were stored at -20^{0}C before being subjected to bioassay analysis.

Microwave extraction (MWE)

This method was referred from Kulkarni and Rathod (2016). Extraction was carried out in microwave/ convection oven (Panasonic NN-CD997S). 10 g of the leaves were mixed with 100 mL of distilled water and were exposed to microwave irradiation for 2, 5 and 8 minutes at medium-low power level (350 W). The extracts were then were filtered and kept at -80^{0}C. The extracts then were dried by using freeze dryer and then stored at -20^{0}C.

Analysis

Total phenolic content (TPC)

TPC of *Mangifera* sp. leaf extracts was analysed using Folin-Ciocalteu colorimetric method described by Gao et al. (2000). About 0.02 mL of 2 mg/mL extract solution was mixed with 0.2 mL Folin- Ciocalteu reagent and 2 mL of distilled water. After 3 minutes, 1 mL of sodium carbonate was added. The mixture was re-incubated for 20 minutes at room temperature. Then, the absorbance was measured at 765 nm using a microplate reader (Fisherbrand™). The total phenolic content was calculated from the gallic acid standard curve. A stock gallic acid standard solution of 1 mg/mL was prepared by dissolving gallic acid in distilled water. The result was expressed as mg gallic acid equivalent per gram of extracts.

Total flavonoid content (TFC)

TFC of sample was determined by using method described by Milan (2011) and Maheshkumar (2012). A stock rutin standard solution of 1 mg/mL was prepared. 0.5 mL of sample extracts, 1.5 mL of 80% methanol, 0.1 mL of 1 M potassium acetate and 0.1 mL of 10% aluminium chloride was mixed. The volume was made up to 5 mL immediately by the addition of distilled water. The solution was incubated for 30 minutes at room temperature. Two hundred microliters of sample extracts were transferred into 96-well plate from centrifuge tube and the absorbance was measured at 415 nm using a microplate reader (Fisherbrand™). The result was expressed as mg of rutin equivalents per gram of samples.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the sample extract was modified according to previous method Mensor et al. (2001) and Dudonne et al. (2009). Stock solution of extracts was prepared as 1 mg/mL in distilled water. The solution was diluted to different concentrations (15.625, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL). A blank solution that served as control was prepared containing ethanol and DPPH. 0.1 mM of DPPH working solution (150 μL) was added to the sample (50 μL) and was mixed in 96-well plates. The mixture was allowed to stand in the dark for 30 minutes. The change in absorbance was measured at 515 nm using microplate reader (Fisherbrand™). Ascorbic acid was used as a standard. Triplicate measurements were carried out and the percentage of DPPH scavenging activity was calculated as follows (Eqn. 1) where, A_c = absorbance of the control and A_s = absorbance of the sample.

$$\text{DPPH scavenging activity (\%)} = [(A_c - A_s)/A_c] \times 100 \qquad \textbf{Eqn. 1}$$

Ferric Reducing Antioxidant Power (FRAP) assay

FRAP assay was determined according to a previous method of Wong et al. (2006) with some modification. About 300 mm acetate buffer was prepared by mixing 0.16 g sodium acetate and 100 mL of 0.28 M glacial acetic acid. 2.4.6-tripyridyl-s-triazine (0.31g) was weighed and dissolved in 100 mL of 40 mm HCl resulting forming of 10 mm 2,4,6-tripyridyl-s-triazine. The working FRAP reagent was prepared by mixing 10 mL of acetate buffer, pH 3.6 with 1 mL of 2,4,6-tripyridyl-s-triazine in 40 mm hydrochloric acid and 1 mL of 20 mm ferric chloride in 10:1:1 ratio and then heated in a water bath at 37^{0}C for 30 minutes. 0.05 mL of sample extract was

mixed with 0.15 mL FRAP reagent. The absorbance was determined at 593 nm immediately and the percentage of inhibition is calculated with the following equation (Eqn. 2) where A_s = absorbance of the sample and A_c = absorbance of the control

$$\text{Inhibition (\%)} = [(A_s - A_c)/A_s] \times 100 \qquad \textbf{Eqn. 2}$$

Data analysis

For each sample, the assay of total phenolic content, total flavonoid content and antioxidant activity by DPPH and FRAP assay were triplicated and calculated as the mean of the measurements. The results were presented with ± standard deviation (SD). One-way ANOVA was used to analyse the effect of extraction methods on the extraction yields, TPC and TFC. Both tests employed Tukey *post hoc* analysis. All statistical analyses were conducted with SPSS software (v.20, SPSS, USA) at a significance level of 0.05.

RESULTS AND DISCUSSION

Extraction yield

The yield of leaf extracts as a result of extracting with different solvents extracts are summarized in Fig. 1. Overall, SXE samples showed the highest percentage yield compared to MWE with 7.1±0.03% (P=0.00), while MWE at 8 minutes extract showed a higher percentage yield compared to other two MWE extract with 6.33±0.05% (P=0.00) (Fig. 1). For MWE extract the extraction yield increased as time exposed to microwave irradiation increased. This result is comparable with previously described by De Castro et al. (2010), that Soxhlet extraction are capable to extracts more sample mass than the majority of the most recent other options such as microwave extraction, supercritical fluid extraction. However, there was no distinct difference in yield of extract between SXE and MWE 8 minutes extract. Previous study by Tan et al. (2014), indicate there were no significant effect of the water-to-powder ratio on the aqueous extraction on the total flavonoid content however, there were no report on extract mass. In addition, the yield also can be influenced by extraction time and temperature, sample-to-solvent ratio and physical characteristics of the sample (Dai & Mumper, 2010).

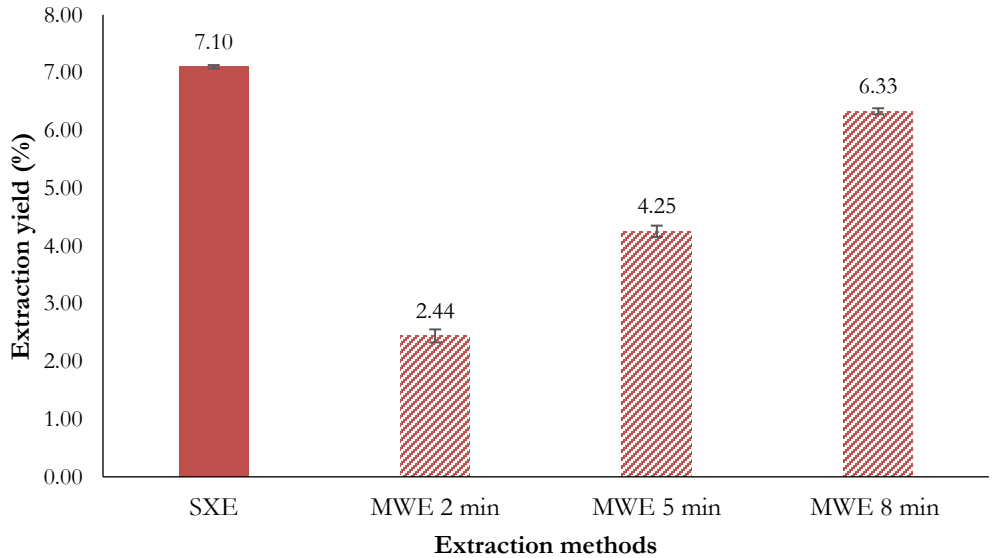

Fig. 1. Extraction yield of *Mangifera* sp. leaf between SXE and MWE.

Total phenolic content

Total phenolic was determined using Folin-Ciocalteu. Folin-Ciocalteu is a colorimetric process based on reactions of electron transfer between the Folin-Ciocalteu reagent and the phenolic compounds. The range of total phenolic content extracted with distilled water were in range 153.19 to 262.13 mg GAE/g. Fig. 2 shows that MWE has the highest phenolic content compared to SXE (259.26 ± 0.03 mg GAE/g) (P=0.863). As for between MWE, MWE 8 minutes extract (262.13 ± 0.05 mg GAE/g) showed a higher phenolic content compared to MWE 5 minutes extract (177.92 ± 0.03 mg GAE/g) and MWE 2 minutes extract (153.19 ± 0.003 mg GAE/g) at P=0.01 and P=0.00 respectively. Based on the result, MWE techniques utilized microwave radiation into heating the solvent (water) and able to break the cellular components of *Mangifera* leaf thus releasing the phenolic compounds in a short time, especially small phenolic molecules. However, the phenolic compounds with higher hydroxyl group might not be able to withstand high heat produce by microwave (Dai & Mumper, 2010). According to Lopez-Arila (2000), MWE can extract phenolic compounds higher compare then other extraction method, and the method precision is significantly better for MWE (e.g. coefficient of variation of 3% for MAE as compared to 15% for Soxhlet and 20% for sonication). Thus, it is in accordance with our finding that MWE can produce higher phenolic content.

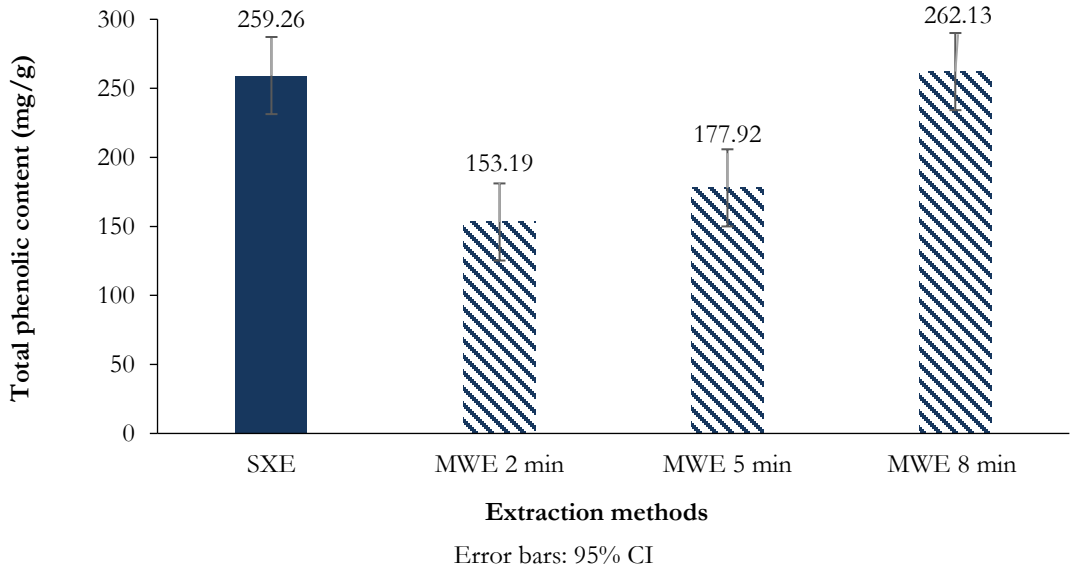

Fig. 2. Total phenolic content in different extraction techniques

Total flavonoid content

Fig. 3 shows the total flavonoid content in samples of *Mangifera* sp. leaves. The range of the total flavonoid in the sample of both extraction techniques was from 234.57 mg rutin/g to 413.46 mg rutin/g. This study used rutin as standard. Rutin is a flavonoid in the flavonol group with a keto group at C-4 and a hydroxyl group on neighboring C-3 or C-5 atoms of flavones and flavonols (Alighiri et al., 2019). Of the four extraction, SXE was observed to have the highest flavonoid content (413.46 ± 0.77 mg rutin/g) compared to MWE at 8 minutes extract (382.62 ± 0.76 mg rutin/g), 2 minutes (334.62 ± 0.54 mg rutin/g) and 5 minutes (234.57 ± 0.34 mg rutin/g) at P= 0.221, 0.00, and 0.00 respectively. In comparison to the previous study by Marjoni et al. (2018), their findings showed that flavonoid content of their *Mangifera indica* leaf extract was higher which is 1240.2 mg QE/g. However, the difference in the flavonoid content could be due to different standards used in measuring the flavonoids. The content of flavonoid can be different regarding their species, growth temperature, and the quality of the soil (Chandra et al., 2014).

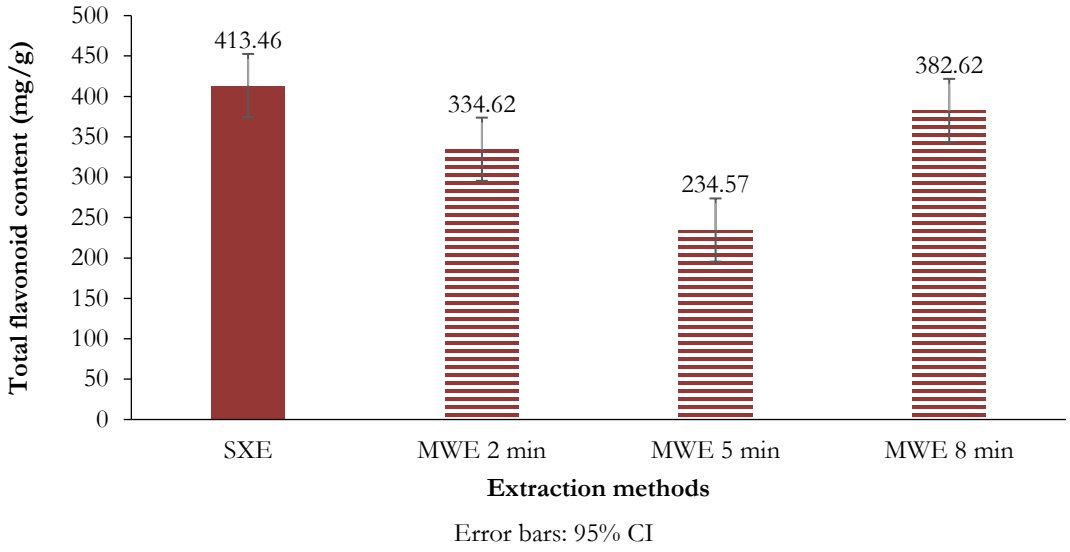

Fig. 3. Total flavonoid content in different extraction techniques

DPPH scavenging assay

According to Husein et al. (2014) the composition of the extracts will influenced the antioxidant activity of the plant extracts. Fig. 4 shows the percentage of scavenging activity of both extractions in samples of *Mangifera* sp. leaves. SXE extract and MWE extract of samples were compared to ascorbic acid which act as standard. The percentage of scavenging activity was performed in the range 0 to 1000 μg/mL for both samples and standard. Based on Fig. 4, the highest maximum scavenging activity can be observed at MWE 8 minutes extract which is 93.57% at a concentration of 250 μg/mL. To support the result, the antioxidant activity from each sample was determined by IC_{50} value. The lowest concentration required to obtain a 50% antioxidant capacity show the extract contains the highest amount of antioxidant. As shown in Table 1, the MWE for 8 minutes and 5 minutes extract revealed highest DPPH scavenging activity, which is 93.57% and 93.06% inhibition at the concentration of 10 μg/mL. However, the MWE at 8 minutes extract possessed the highest percentage of scavenging activity at only 10 μg/mL. These overall results indicated that MWE at 8 minutes extract showed the best scavenging activity than SXE.

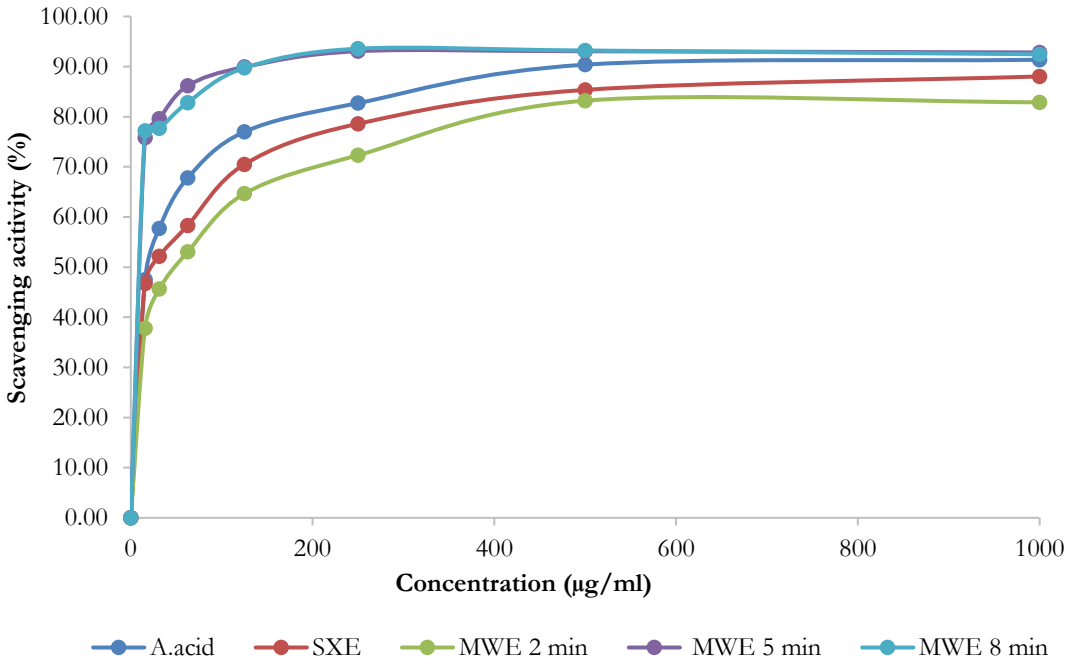

Fig. 4. Percentage of scavenging activity of SXE and MWE compared to ascorbic acid (standard).

Table 1. IC_{50} and maximum inhibition concentration for standard, SXE and MWE.

Sample	IC_{50} value (µg/mL.)	Maximum inhibition concentration (%)
Ascorbic acid (standard)	20±0.45	91.35±0.03
SXE	25±0.36	88.03±0.06
MWE 2 minutes	50±0.43	83.2±0.17
MWE 5 minutes	10±0.05	93.06±0.01
MWE 8 minutes	10±0.06	93.57±0.00

IC_{50} = inhibitory concentration at which 50% radical are scavenged

Ferric Reducing Antioxidant Power (FRAP)

Fig. 5 shows the percentage of reducing activity is based on the ability of the antioxidants contained in the extracts to reduce Fe^{3+}- TPTZ complex to a blue coloured Fe^{2+}- TPTZ at low pH. MWE at 8 minutes extract show the highest inhibition percentage in reducing activity compared to SXE and other two MWE extract. The capacity of reducing activity was determined by IC_{50} value from each sample. The maximum reduction activity was achieved by MWE at 8 minutes extract which is 60% at the concentration of 110±0.52 µg/mL. Comparison between sample and standard (ascorbic acid), ascorbic acid showed the highest ability in reducing FRAP activity, which measured by the lowest IC_{50} value. Nonetheless, based on the focus of this study MWE 8 minutes extract posed lowest IC_{50} compared to the other two MWE and SXE.

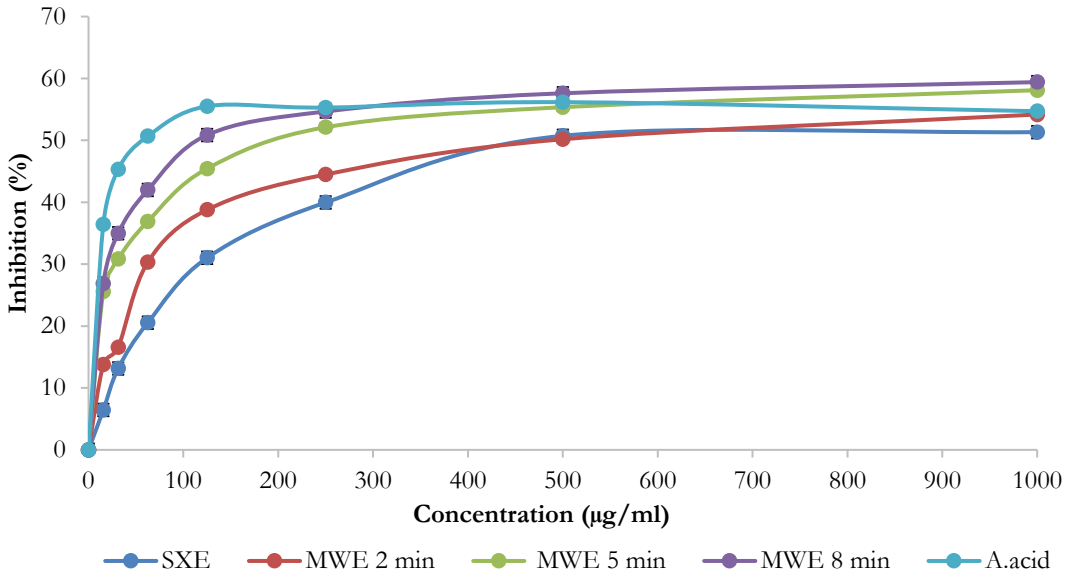

Fig. 5. Percentage of FRAP inhibition for SXE and MWE compared to ascorbic acid (standard).

Table 2. IC_{50} and maximum inhibition concentration for standard, SXE and MWE.

Sample	IC_{50} value (µg/mL.)	Maximum inhibition concentration (%)
Ascorbic acid (standard)	55±0.51	56.17±0.49
SXE	460±0.17	51.33±0.09
MWE 2 minutes	460±0.15	54.18±0.08
MWE 5 minutes	190±0.61	58.11±0.50
MWE 8 minutes	110±0.52	59.41±0.44

IC_{50} = inhibitory concentration at which 50% radical are scavenged

CONCLUSION

In conclusion, both *Mangifera* sp. leaf crude extract, Soxhlet and microwave showed positive antioxidant activities. There are no distinct differences in yield, total phenolic content and total flavonoid content between Soxhlet extract and MWE at 8 minutes. MWE showed that it can be used in samples extraction as it produced a higher yield in a shorter time compare to SXE and it did give better scavenging and reducing activities.

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