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Total Flavonoids, Total Phenolic Content and Antioxidant Activity from Fruits, Leaves, Twigs and Flowers of *Mesua ferrea* L.

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Abstract

The objective of this study was to compare antioxidant activity from crudes of fruits, leaves, twigs and flowers of *Mesua ferrea* L. with hexane, dichloromethane and methanol by controlling time and temperature in the solvent extraction. All crude extracts were investigated for their antioxidant capacity in 1,1-diphenenyl-2-picryhydrazyl (DPPH) radical scavenging. The results indicated that the high polar solvent exhibited the highest DPPH radical scavenging activity. The methanol extracts of leaves show the highest activity with IC_{50} of 89.94±0.65 µg/mL. The flowers, twigs and fruits methanol crude extracts show IC_{50} of 94.26±5.93, 94.45±1.67 and 722.94±5.85 µg/mL, respectively. The methanol extracts of leaves also had the highest total flavonoids content (325.79±3.08 mgQE/g). The methanol extract of flowers and the dichloromethane extract of fruits presented higher total phenolic contents than the other extracts which contained 769.11±46.64 mgGAE/g and 703.62±12.62 mgGAE/g, respectively, followed by the methanol extract of leaves (348.36±38.53 mgGAE/g).

Keywords: Total flavonoids content, total phenolic content, antioxidants, *Mesua ferrea* L.

Introduction

Over the years, free radicals are great interest because they affect human health reacting with biomolecules in the body. It may cause defects in biomolecules, destroy DNA, and change proteins and fats in the cell membrane, constructing covalent bonds between proteins causing the protein to malfunction. The oxidative reactions of free radical in the body are a danger to the cardiovascular system, immune system and the brain [1]. This is a major cause of disease or induction of many diseases such as heart disease, high blood pressure, cancer, diabetes, aging and degeneration of the nervous system. The molecules are collectively known as reactive oxygen species and can damage the body causing abnormal metabolic processes leading to age-related disorders, to wrinkles or severe disease such as intellectual deficiencies, Parkinson's disease and cancer [2]. The benefits are due to the presence of polyphenols, flavonoids, carotenoids, and vitamins [3].

Substances used against or to inhibit free radicals are called antioxidants which they regulate free radical, not to stimulate oxidative reaction, not destroying cellular component. Antioxidants contain natural substances, such as amino acids, vitamin C, carotene, etc. Parts of fruits, leaves, twigs, flowers, bark and root of herbal plants have many active compounds like flavonoids, polyphenols and tannin. Medicinal plants have been investigated for their antioxidant activities and ability to reduce oxidative damage. In recent years, phenolic compounds have received attention because of their antioxidant and biological properties to human health [4]. Antioxidant substances of plants are of high importance, and might be effective as therapeutic agents in the treatment of various diseases [5]. It plays an important role

in the treatment of human neurodegenerative disorders like diabetes, inflammation, Alzheimer's disease, autoimmune pathologies, and digestive system disorders [6].

Mesua ferrea L. is in the family Calophyllaceae. It is native plant in India, Sri Lanka, Myanmar, Thailand, Malaysia and Singapore. It is about 15 - 25 m but can be up to 30 m in height. A large shrub like a pagoda, there are a few puffs at the base. It is a deciduous wood, hardwood branches and slender stalks hanging down. The bark is dark brown, shallow cracks and slightly yellowish latex. The leaves are single cotyledons and spear shaped. The flowers are a single flower or a pair of flowers on the branches. The petals are white to light yellow with 5 petals overlapping. The flower is yellow, orange and fuzzy. The blooming flowers fall in summer and rainy season. The fruit is ovate, very strong and orange or purple in color. In addition, *Mesua ferrea* L. is also a regular plant in Phichit Province and its flower is a regular flower of Nakhon Si Thammarat Rajabhat University.

Studies on the *in vivo* immunomodulatory activity of mesuol essential oils, which were isolated from the seeds of *Mesua ferrea* L. found that they evoked a significant dose dependent increase in antibody titer values in cyclophosphamide. Mesuol potentiated percentage neutrophil adhesion in neutrophil adhesion test in rats and phagocytosis in a carbon clearance assay [7]. The study of free radicals is made up of oxygenation processes in living organisms. The antioxidant defense system can break down biomolecules within the organism's system. The stem bark of *Mesua ferrea* L. was extracted using chloroform and ethanol. Both extracts showed significant antioxidant activity. The chloroform extract showed the protective effect against H_2O_2 induced oxidative stress of human erythrocytes (IC_{50} 1001±3.097 μg/mL) while ethanol extract showed protective effect (IC_{50} 534.3±3.680 g ml^{-1}) significantly equivalent to butylated hydroxyl anisole. Moreover, HPLC showed that good oxidant compounds such as gallic acid, ellagic acid, coumaric acid, vanillic acid, rutin, quercetin, myricetin and kaempferol [8].

Mesua is a small genus with 17 species. The hexane extract from the bark of *Mesua elegans* showed a significant acetylcholinesterase inhibitory activity. Nine of 4-phenylcoumarins can be isolated. Mesuagenin B showed the most potent inhibitory activity with an IC_{50} of 0.7 μM [9].

Based on previous data, it is interesting to study the antioxidant activity of other parts of *Mesua ferrea* L. as a basic information for further pharmaceutical use. The objectives in this research were to determine the total flavonoids and total phenolic content in various extracts of *Mesua ferrea* L. using spectrophotometry, as well as to examine antioxidant activity of the extracts using an *in vitro* system.

Materials and methods

Chemicals and instrument

Hexane, dichloromethane and methanol were purchased from Merck Ltd. Standards of phenolic content (gallic acid), flavonoids content (quercetin), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, aluminium chloride ($AlCl_3$), sodium carbonate (Na_2CO_3) and potassium acetate (CH_3COOK) were obtained from Merck Ltd. All solvents and chemicals were of analytical grade. The absorbance of samples were measured with a UV-Vis spectrophotometer (Spectroquant® Prove 300).

Plant material

The fruits, leaves, twigs and flowers of *Mesua ferrea* L. were collected in July 2015 from Nakhon Si Thammarat Rajabhat University, Thangew, Muang, Nakhon Si Thammarat Province. Samples of plant material were given to the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmacy, Prince of Songkla University for identification herbarium specimen in SKP 216 13 06 01.

Preparation of plant extracts

The plant samples were chopped and ground in a mechanical blender. The extracts were prepared by continuous solvent extraction of 10 g of ground sample with 50 mL of hexane in room temperature for 3 days (each 3 days × 2 times). The solvent was evaporated under reduced pressure to give hexane extract. After the solution and the residue were each isolated, the residue was further immersed in dichloromethane and methanol at room temperature continuously (each 3 days × 2 times). The process of extraction is shown in **Figure 1**.

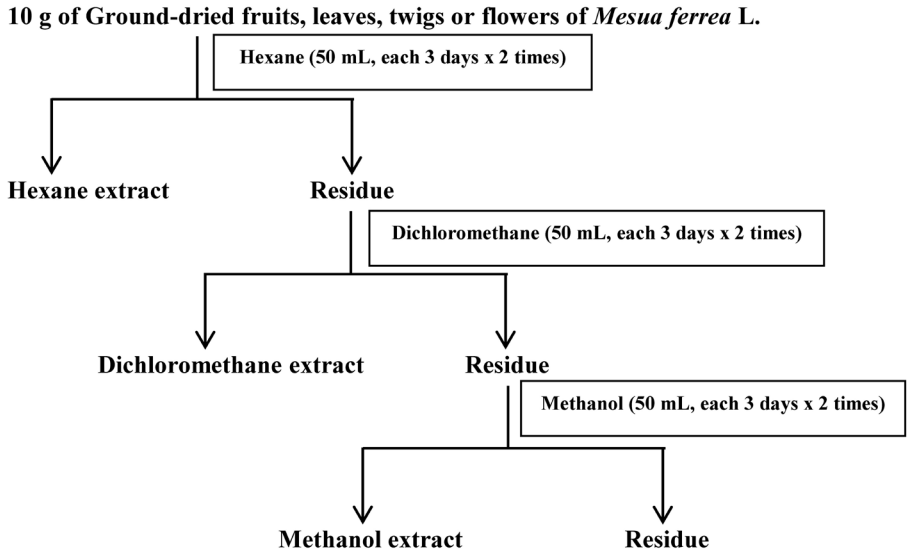


Figure 1 Continuously solvent extraction of *Mesua ferrea* L.

Evaluation of antioxidant activity

A DPPH solution (0.3 mM) was mixed with sample solutions at different concentrations. A control containing ethanol and DPPH solution was also realized. All solutions were incubated for 1 h at room temperature. The absorbance of the solution was subsequently measured at 517 nm by UV-Vis spectroscopy.

$$\% \text{ inhibition} = \left[\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right] \times 100 \quad (1)$$

The percentage of DPPH reduced was plotted against the concentration of each sample (31.25 - 500 ppm for hexane and dichloromethane extracts and 31.25 - 125 ppm for methanol extract and ascorbic acid). An IC_{50} value which was defined as the concentration of the sample needed to scavenge 50 % of DPPH, was calculated from the graph.

Determination of total phenolic content

The total phenolic content (TPC) was determined by using Folin-Ciocalteu reagent. Gallic acid was used as a standard for the experiment. Briefly, 0.5 mL of crude extract (1 mg/mL) was mixed with 2.5 mL of 10 % Folin-Ciocalteu reagent. After 5 min, 2 mL of 7.5 % Na_2CO_3 was added. The mixture was allowed to stand for 1 hr in the dark, and absorbance was measured at 765 nm. The total phenolic content was calculated from the calibration curves of gallic acid, and the results were expressed as mg of gallic acid equivalent (GAE) per g of plant sample using the following formula;

$$\text{Total phenolic content (TPC)} = \frac{GAE \times V \times D}{W} \quad (2)$$

where V is the volume of the extract solution in mL, W is the weight of the plant sample in grams and D is the dilution factor.

Determination of total flavonoids content

The aluminium chloride colorimetric method was used to determine the total flavonoids content (TFC) in crude extract. Briefly, 0.5 mL of crude extract (5 mg/mL) was mixed with 2 mL in appropriate solvent, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M potassium acetate and diluted with distilled water in a 5 mL volumetric flask. The absorbance was determined at 415 nm. The data of total flavonoids content were expressed as mg of quercetin equivalents/g of plant sample.

Statistical analysis

Data were analyzed using the SPSS program for Windows (version 16.0). Data were expressed as mean ± SD (n = 3), if justified by the statistical probability ($P < 0.05$), by Tukey one-way ANOVA test.

Results and discussion

The yields of the extract obtained per 10 g of dry fruits, leaves, twigs and flowers with the different solvents are given in **Table 1**.

Table 1 The yields of the extracts obtained by the different solvents.

Sample	Weight of sample (g)	Hexane extracts		Dichloromethane extracts		Methanol extracts	
		Weight (g)	%yield	Weight (g)	%yield	Weight (g)	%yield
Fruits	10.00	0.3225	3.22	0.3611	3.61	0.7310	7.31
Leaves	10.00	0.6450	6.45	0.7071	7.07	0.2399	2.40
Twigs	10.00	0.2630	2.63	0.0790	0.79	0.3364	3.36
Flowers	10.00	0.9722	9.72	0.8202	8.20	0.7250	7.25

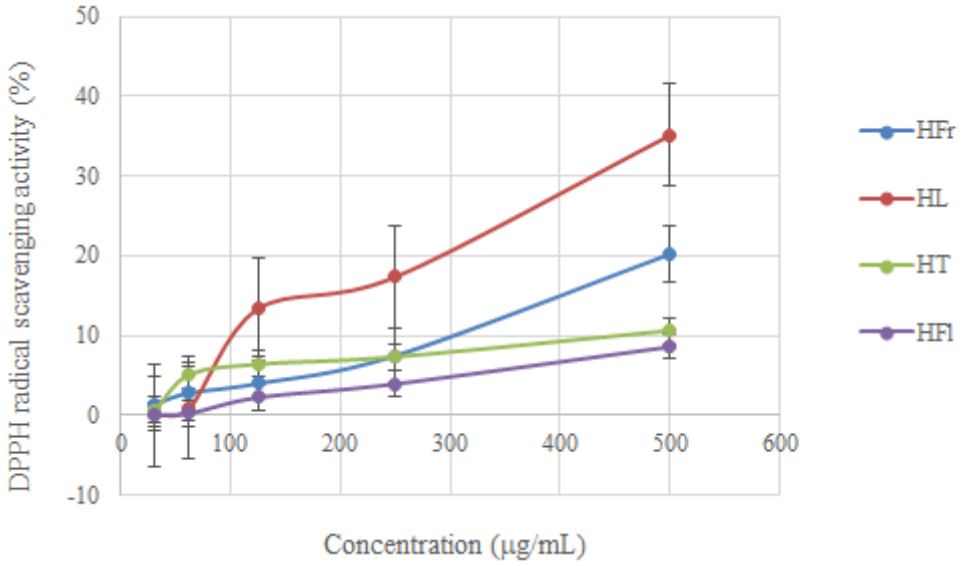
The highest yields of the fruits and twigs extracts were obtained by extraction with methanol at 7.31 and 3.36 %, respectively. The yields of leaves extract was the highest by dichloromethane at 7.07 %, while the flowers show the highest yields by hexane extracts.

The antioxidant activities of each extract were analyzed. The DPPH method was determined by sample preparation at concentrations of 31.25, 62.5, 125, 250 and 500 µg/mL and measured for wavelength 517 nm using Spectrophotometer compared with ascorbic acid standard solutions. The purple color of DPPH free radicals after reaction with antioxidant gave a yellowish color. Three replications were performed at each concentration. The results are shown in **Table 2**. Plotting the relationship between % DPPH radical scavenging activity and concentration to compare the effectiveness of antioxidant interactions between different parts are shown in **Figure 2**.

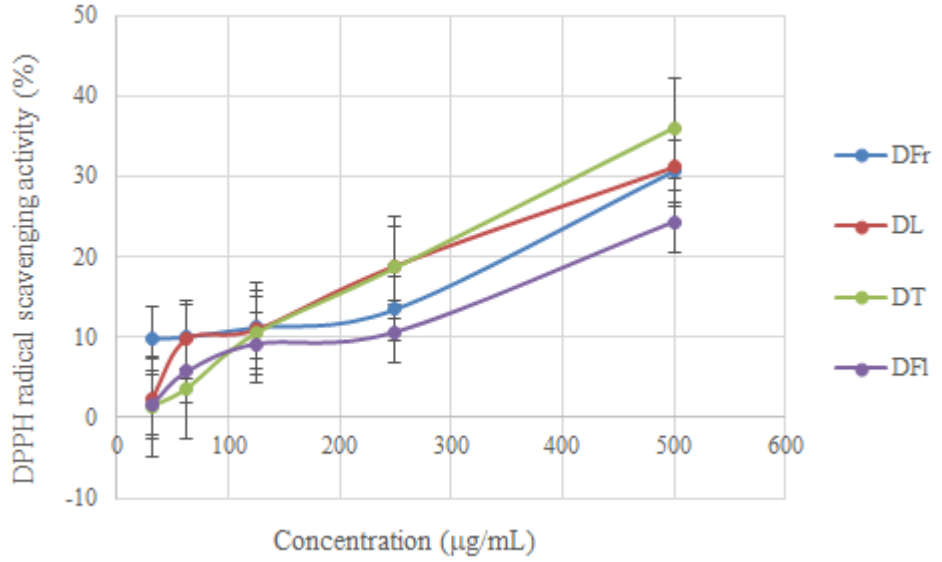
Table 2 Antioxidant activity of different concentrations of hexane, dichloromethane and methanol extracts of *Mesua ferrea* L. on DPPH radical scavenging.

Crude extracts	Concentration (µg/mL)	%inhibition			
		Fruits (Fr)	Leaves (L)	Twigs (T)	Flowers (Fl)
Hexane (H)	31.25	1.43±0.09	0.05±0.00	0.61±0.08	0.10±0.08
	62.5	2.81±0.02	0.89±0.12	5.11±1.13	0.24±0.06
	125	4.00±0.12	13.34±0.06	6.42±0.87	2.25±0.12
	250	7.49±0.07	17.36±0.06	7.36±1.15	3.96±0.19
	500	20.22±0.08	35.12±0.10	10.64±0.32	8.60±0.12
Dichloromethane (D)	31.25	9.77±0.29	2.35±0.21	1.43±0.36	1.61±0.18
	62.5	10.04±0.09	9.76±0.37	3.66±0.26	5.72±0.58
	125	11.20±0.63	10.96±0.03	10.50±1.57	11.33±2.26
	250	13.48±0.10	18.87±0.12	18.67±0.35	10.68±0.79
	500	30.61±0.35	31.13±0.15	36.02±0.15	24.37±2.33
Methanol (M)	31.25	2.20±0.24	18.23±0.41	15.76±0.72	4.35±0.42
	62.5	2.34±0.20	30.72±0.27	44.42±0.17	33.74±5.77
	125	8.06±0.08	71.45±0.31	60.82±1.03	69.08±2.85
	250	17.07±0.12	88.88±1.00	75.49±0.87	85.04±2.12
	500	33.94±0.07	92.30±0.25	93.16±0.30	91.78±0.39

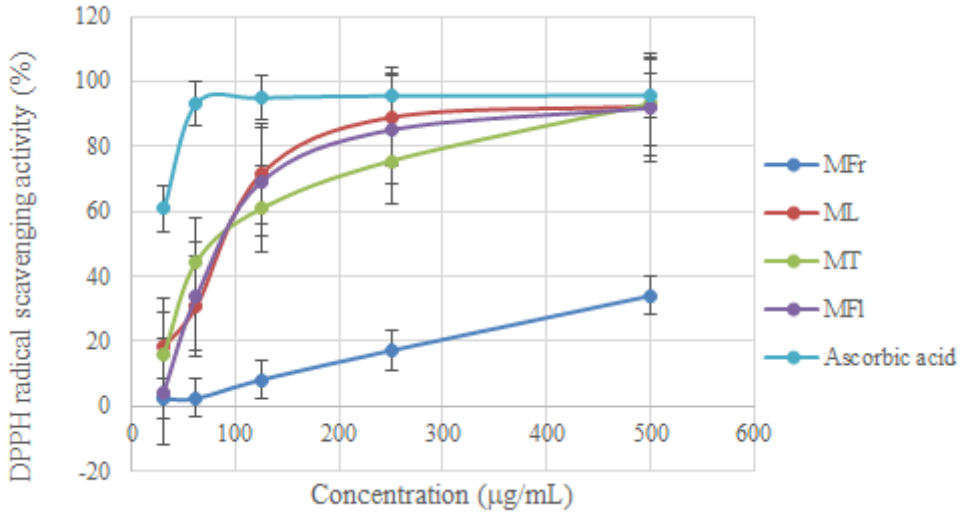
Results are presented as means ± SD (n = 3)



(a)



(b)



(c)

Figure 2 Average DPPH radical scavenging activity of *Mesua ferrea* L. extracts compared with ascorbic acid a) Hexane extract b) Dichloromethane extract c) Methanol extract.
(HFr: Hexane extract of fruits, DFr: Dichloromethane extract of fruits, MFr: Methanol extract of fruits, HL: Hexane extract of leaves, DL: Dichloromethane extract of leaves, ML: Methanol extract of leaves, HT: Hexane extract of twigs, DT: Dichloromethane extract of twigs, MT: Methanol extract of twigs, HFl: Hexane extract of flowers, DFl: Dichloromethane extract of flowers and MFl: Methanol extract of flowers)

It was found that the hexane extract exhibited relatively low antioxidant activity same as in crude extract from dichloromethane. The crude extract of dichloromethane from fruits, twigs and flowers tended to be better than the hexane extracts. The methanol extracts of various parts exhibited effective antioxidant activity. The leaves, flowers and twigs showed high performance for radical inhibition, while the fruits exhibited low level activity. In comparison, the efficiency of the antioxidant activity of various solvents was found that the extracts from the fruits showed similar effects in all 3 solvents, while in the methanol crude extracts from leaves, twigs and flowers showed better inhibitory efficacy than the other solvents. Determination of the inhibitory inhibition 50 % of each extracts compare with ascorbic acid standard solution (IC_{50} = 24.28±4.28 µg/mL) showed that methanol extracts of leaves, flower and twigs exhibited high antioxidant properties with IC_{50} of 89.94±0.65, 94.26±5.93 and 94.45±1.67 µg/mL, respectively (**Table 3**).

Table 3 Antioxidant activity of *Mesua ferrea* L. extracts by DPPH method.

Crude extracts		IC_{50}, µg/mL
Fruits	Hexane	1275.59±22.88^{e}
	Dichloromethane	943.40±17.64^{d}
	Methanol	722.94±5.85^{b}
Leaves	Hexane	807.84±1.23^{c}
	Dichloromethane	690.55±2.57^{b}
	Methanol	89.94±0.65^{a}
Twigs	Hexane	2824.23±87.34^{g}
	Dichloromethane	684.34±1.44^{b}
	Methanol	94.45±1.67^{a}
Flowers	Hexane	2466.35±10.71^{f}
	Dichloromethane	937.34±29.43^{d}
	Methanol	94.26±5.93^{a}
Ascorbic acid		24.28±4.28

$^{a-g}$ significant ($p < 0.05$) differences within a column.
Results are presented as means ± SD (n = 3)

Phenolic compounds are secondary metabolites in plants. They are known to have antioxidant activity [10]. The results obtained in this study showed a significant level of phenolic compounds in hexane, dichloromethane and methanol extracts of the fruits, leaves, twigs and flowers of *Mesua ferrea* L. (**Table 4**). The methanol extract of flowers and the dichloromethane extract of fruits presented higher contents than the other extracts consistent with the methanol extract of *Pasiflora* which contains constituents with significant phenolic content and antioxidant properties [11].

Table 4 Total phenolic content of *Mesua ferrea* L. extracts.

Crude extracts		Total phenolic content, mgGAE/g
Fruits	Hexane	111.62±13.24^{de}
	Dichloromethane	703.62±12.62^{a}
	Methanol	47.91±28.67^{ef}
Leaves	Hexane	139.40±14.69^{d}
	Dichloromethane	83.33±0.76^{def}
	Methanol	348.36±38.53^{b}
Twigs	Hexane	264.02±47.97^{c}
	Dichloromethane	88.69±23.55^{def}
	Methanol	290.52±8.09^{bc}
Flowers	Hexane	73.90±2.68^{def}
	Dichloromethane	23.19±5.53^{f}
	Methanol	769.11±46.64^{a}

$^{a-f}$ significant ($p < 0.05$) differences within a column.
Results are presented as means ± SD (n = 3)

Flavonoids are one class of secondary plant metabolites that are also known as vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colors of plants [12]. Flavonoids are ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activities [13]. The total flavonoids content of *Mesua ferrea* L. extract was also determined using the aluminium chloride colorimetric method (**Table 5**). The results were expressed as mg of quercetin equivalents/g of plant sample. Leaves flavonoid content was higher than fruits, twig and flowers. Methanol extracts of leaves and flowers showed more flavonoids than low polar extracts. The methanol extracts of leaves showed the most abundant of total flavonoids contents (325.79±3.08 mgQE/g). Secondly, the dichloromethane extract of the fruits and methanol extract of the leaves exhibited a total flavonoid content of 274.33±25.14 and 267.04±12.22 mgQE/g, respectively. The high level of total flavonoid in the methanol extract of *Mesua ferrea* L. is consistent with the highest amounts of total flavonoid content which were found in the methanol extract of *Trifolium pratense* L. (red clover) [14]. The polarity of the extraction solvent was correlated with the total phenolic content, total flavonoids content and antioxidant activity [15].

Table 5 Total flavonoids content of *Mesua ferrea* L. extracts.

Crude extracts		Total flavonoid content, mgQE/g
Fruits	Hexane	86.62±15.21^{d}
	Dichloromethane	274.33±25.14^{b}
	Methanol	18.92±0.36^{fg}
Leaves	Hexane	142.46±25.97^{c}
	Dichloromethane	28.71±0.95^{efg}
	Methanol	325.79±3.08^{a}
Twigs	Hexane	50.38±10.33^{def}
	Dichloromethane	58.92±14.06^{de}
	Methanol	49.12±10.66^{def}
Flowers	Hexane	35.58±7.24^{efg}
	Dichloromethane	3.71±0.95^{g}
	Methanol	267.04±12.22^{b}

$^{a-g}$ significant ($p < 0.05$) differences within a column.
Results are presented as means ± SD (n = 3)

The methanol extracts of *Mesua ferrea* L. showed significant antioxidant activity when compared to the respective standards. The hydrophilic solvent generally showed more active compounds than the hydrophobic solvent. Polyphenols and flavonoids have been reported to exhibit a wide range of biological activities. Further phytochemical studies can be done for the isolation of compounds from the methanol extracts. The leaves of *Mesua ferrea* L constitute a natural source of potent antioxidants that may prevent many diseases and could be potentially used in pharmaceutical industries.

Conclusions

The study reveals that the leaves of *Mesua ferrea* L. in methanol extracts showed the highest antioxidant activity and total flavonoids content. In addition, it was established that high phenolic content could be recovered. The methanol extract of flowers showed the most of total phenolic content which might be principally responsible for their strong antioxidant activities.

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