INVESTIGATION OF BIOACTIVE SUBSTANCES CONTENTS AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF *MICHELIA CHAMPACA* L. (SAGAWA) FLOWER

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Abstract

The objective of this study was to examine the yield percent of organic solvent extractable matter, the content of bioactive substances (total phenolic contents and total flavonoids contents) and free radical scavenging activity of ethanolic and watery extracts of *Michelia champaca* L. (Sagawa flower). The results indicated that *M. champaca* was more soluble in polar solvent and it had pronounced effects on both phenolic compound levels and antioxidant potential. Ethanolic extract contained the higher amounts of bioactive compounds and exhibited the better antioxidant activity than watery extract. The evaluation of antioxidant activity of both extracts revealed highly significant correlation between anti-radical ability and total phenolic and total flavonoid contents.

Keywords: *Michelia champaca* L., total phenolic contents, total flavonoids contents, antioxidant activity

Introduction

Michelia champaca L. (Figure 1) is commonly known as Sagawa in Myanmar and it is a tall tree with yellow fragrant blossoms. It is commonly used in many traditional herbal preparations. The plant is also reported to have significant wound healing, antimicrobial, antidiabetic, antitumor, anti-inflammatory, antioxidant and anti-infective properties. Phenolics possess a good spectrum of biochemical activities like antioxidant, anti-mutagenic, anti-carcinogenic likewise ability to change the organic phenomenon (Sulaiman, 2014). Phenolics are the biggest group of phytochemicals that account for many of the antioxidant activity in plants or plant products. Since the phenolic compounds possess the strong evidence of biological activities, Biju *et al.* (2013) focused on determination of total phenolic content in the selected medicinal plant. Sagawa flower could also be a crucial herbal drug with some important marker useful to treat some challenging diseases to marking in future life. The herbal or traditional medicine involves the use of different types of organic extracts or bioactive chemical constituents. This type of biochemical investigation provides health care at an affordable cost. So, the Sagawa flower had been chosen to investigate some bioactivities in this research paper.

Figure 1 Photographs of plant and flowers of *Michelia champaca* L. (Sagawa)

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Materials and Methods

Sample Collection and Preparation of *M. champaca* (Sagawa) Flower

Flowers of *M. champaca* (Sagawa) were collected from Mandalay Region in June, 2017. Then, the sample was authenticated at the Department of Botany, University of Yangon. The collected sample was washed with water and dried in an oven at 50 °C. The dried pieces were made into powder by using grinding machine. The powdered sample was stored in air-tight container to prevent moisture changes and other contaminations.

Preparation of Different Extracts of *M. champaca* (Sagawa) Flower

The powdered sample was extracted by cold maceration for 72 h at 37 ℃ with occasional shaking with different solvents like petroleum ether, ethyl acetate and ethanol to ensure complete extraction. After this, the extracts were filtered through Whatman filter paper and the extracts were collected and stored at 4 ℃ in the refrigerator. Watery extract was prepared by boiling 100 g of sample with 500 mL of distilled water for 6 h and filtered. It was repeated three times and the filtrates were combined, followed by heating on water bath and sand bath to obtain watery extract. The percentage yield of various extracts of the flower of *M. champaca* was calculated.

Determination of Total Phenolic Contents of *M. champaca* (Sagawa) Flower by FCR Method

One of the antioxidative factors, total phenolic contents (TPC) of watery and ethanol extracts were measured spectrophotometrically according to the Folin-Ciocalteu method (Saxena *et al.,* 2013). Firstly, the sample solution was prepared by dissolving 2 mg of each extract in 100 mL of distilled water respectively. 0.5 mL of each of the sample solution and these solutions were mixed with 0.5 mL of methanol. 5 mL of FCR reagent (1:10) was added to each of the sample solution and these solutions were incubated for 5 min. 4 mL of 1 M sodium carbonate solution was added to each tube and kept at room temperature for 2 h. Then, the UV absorbance of reaction mixture was read at λ_{max} 765 nm. The blank solution was prepared as the above procedure by using distilled water instead of sample solution. Total phenolic content was estimated as μg gallic acid equivalents per mg of different extracts (μg GAE/ mg).

Determination of Total Flavonoids Contents by Aluminium Chloride Colorimetric Assay

The total flavonoids contents (TFC) of the samples were determined by UV spectrophotometer according to the aluminium chloride colorimetric assay (Zhishen *et al.,* 1999). Each extract (10 mg) was mixed with 20 mL of distilled water to get 500 μg/mL solution. Each extract solution (0.5 mL) was mixed with 1.5 mL of methanol, 0.1 mL of 1 % aluminium chloride solution, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The resultant mixture was allowed to stand for 30 min at room temperature. Absorbance of the resulting solution was measured against reagent blank solution at 415 nm by using a spectrophotometer (UV-7504, China). The experiment was done in triplicate. The concentration of quercetin equivalent (QE) in the dried flower extracts was calculated by using the linear regression equation from standard calibration curve of quercetin. Total flavonoids contents (TFC) of the dried flower sample were expressed as mg quercetin equivalent per 1 g dried flower extract (mg of QE/g).

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

The free radical scavenging activity of watery and ethanol extracts of *M. champaca* (Sagawa) flower was measured by using DPPH free radical scavenging assay (Marinova and Batchvarov, 2011). The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was also prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated

at room temperature and were shaken on shaker for 30 min. After 30 min, the absorbance of different concentrations (12.5, 25, 50, 100, 200 and 400 μg/mL) of tested sample was measured at 517 nm using UV-7504 spectrophotometer. Absorbance measurements were done in three times for each concentration and the mean value so obtained were used to calculate percentage of radical scavenging activity as shown in Table 4. The antioxidant power (IC_{50}) is expressed as the concentration (μg/mL) of test substances that results in a 50 % reduction of initial absorbance of DPPH solution. IC_{50} (50 % inhibition concentration) values were calculated by linear regressive excel program.

Results and Discussion

The phytochemical constituents possess varying degrees of antioxidant activity. Hence, they can be assumed as health protective agents. It has been reported the presence of alkaloids, α-amino acids, carbohydrates, organic acids, flavonoids, glycosides, phenolic compounds, reducing sugars, saponin, starch, steroids, tannins and terpenoids, except harmful cyanogenic glycosides, and ten compounds present in essential oil extracted from Sagawa flower identified by GC-MS analysis (Myint Myint Khin, 2021).

Percentage Yields of Different Extracts of *M. champaca* (Sagawa) Flower

Extraction involves the use of an inert solvent which actively separates the molecules from the plant's parts. The extracts obtained with various solvents such as petroleum ether, ethyl acetate, ethanol and distilled water were weighed and their percentage yields were calculated as compared to the initial weight of the plant material to get the extractive values. The experimental data are presented in Table 1. The present study revealed that the yield of watery extract (11.49 %) showed the highest value, followed by the yield of ethanol extract (6.81 %), ethyl acetate extract (2.08 %) and petroleum ether extract (0.78 %). The resulted data suggested that the extractable matter contents increase with increase in polarity of the solvents. These data indicated that the amounts of polar constituents were higher than that of non-polar constituents in the Sagawa flower.

Table 1 Percentage Yields of Different Extracts of *M. champaca* (Sagawa) Flower

No.	Samples	Percentage yields (%)
1.	Watery extract	11.49
2.	Ethanol extract	6.81
3.	Ethyl acetate extract	2.08
4.	Petroleum ether extract	0.78

Total Phenolic Contents of Crude Extracts of *M. champaca* (Sagawa) Flower

The phenolic compounds are plant metabolites characterized by the presence of several phenol groups. Some of them are very reactive in neutralizing free radical by donating a hydrogen atom or an electron (Almey *et al.,* 2010). Phenolic compounds have antioxidant properties of protective against degenerative disease like heart diseases and cancer. The total phenolic contents of water and ethanol crude extracts of flowers of Sagawa were evaluated with spectrophotometric method using Folin-Ciocalteu reagent. The principle of this method is based on the reduction ability of the phenol functional group. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions resulting in the formation of blue coloured complex (Dudonee *et al.,* 2009).

The reduction of complex will increase when the extracts contain more phenolic compounds. Thus, the colour will be darker and the absorbance will be higher. The absorbance can be measured at UV 765 nm. Gallic acid (3, 4, 5-trihydroxybenzoic acid) was used to construct standard calibration curve. Total phenolic content was expressed as microgram gallic acid equivalent per milligram (µg GAE/mg) of crude extract (Saxena *et al.,* 2013). In this study, high phenol contents have been found to exert high antioxidant potential. The study shows a direct relation between antioxidant activity and total phenol contents. According to the results as shown in Table 2, the higher TPC (µg GAE/mg) was detected in ethanol extract (508.95 µg GAE/mg) than watery (328.55 µg GAE/mg) extract of Sagawa flowers. This means that phenolic compounds were more soluble in ethanol. Comparison of TPC in watery and ethanol extracts of Sagawa flower is represented by a bar graph in Figure 2.

Table 2 Total Phenolic Contents in Watery and Ethanol Extracts of *M. champaca* (Sagawa) Flower

Extracts	TPC (µg GAE/mg ± SD)
Watery	328.55 ± 0.0052
Ethanol	508.95 ± 0.0043

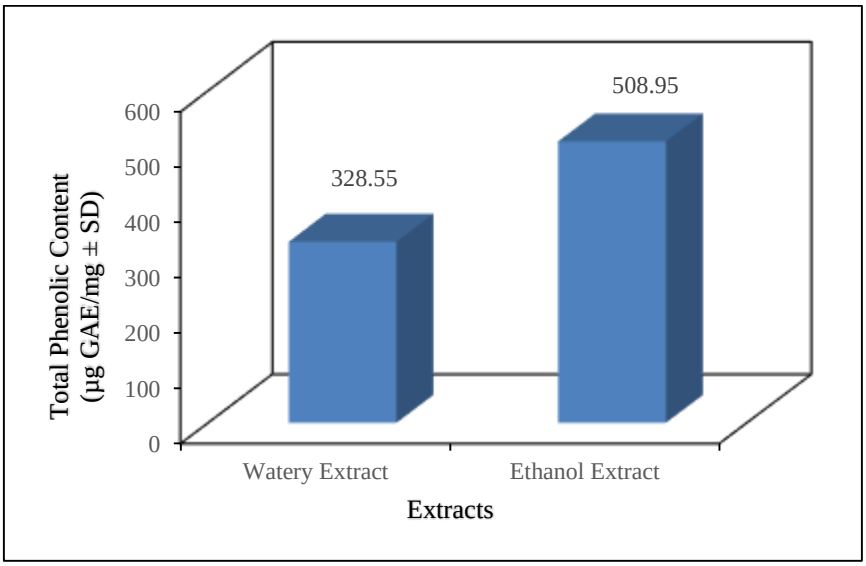

Figure 2 Total phenolic contents of watery and ethanol extracts of *M. champaca* -(Sagawa) Flower

Total Flavonoids Contents of Crude Extracts of *M. champaca* (Sagawa) Flower

The total flavonoids contents of the watery and ethanol crude extracts of the flowers of Sagawa were evaluated with spectrophotometric method using aluminium chloride reagent. As shown in Table 3 and Figure 3, the higher TFC (mg GAE/g) was detected in ethanol (9.63 mg QE/g) than watery (0.88 mg QE/g) extracts of Sagawa flower. This means that flavonoid compounds were more soluble in ethanol. In this study, high phenol contents have been found to exert high flavonoid content. Similar to phenolic content, ethanolic extract had higher flavonoid content compared to watery extract.

Table 3 Total Flavonoids Contents of Watery and Ethanol Extracts *M. champaca* (Sagawa) Flower

Extracts	TFC (mg of QE/ g of extract ± SD)
Watery	0.88 ± 0.002
Ethanol	9.63 ± 0.001

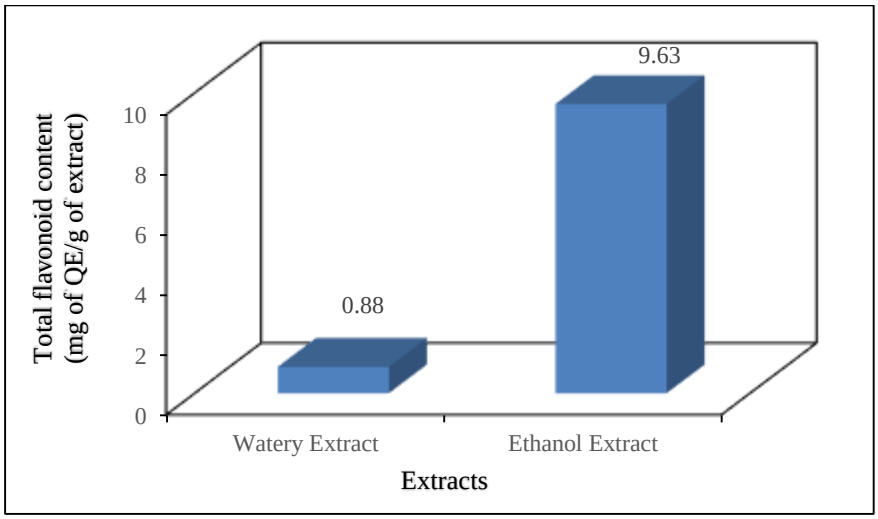

Figure 3 Total flavonoids contents of watery and ethanol extracts of *M. champaca* (Sagawa) Flower

Radical Scavenging Activity of Crude Extracts of Flower of *M. champaca* (Sagawa) Flower by DPPH Radical Scavenging Assay

Antioxidant compounds in plant play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. The antioxidant activity of watery and ethanol crude extracts of Sagawa flowers was evaluated by the DPPH (2,2-diphenyl-1 -picrylhydrazyl) radical scavenging assay. Butylated hydroxytoluene was used as a standard. Colorimetry with DPPH, a stable free radical, has been reported as a simple method for evaluation of the free radical scavenging activity. It tends to capture hydrogen from the antioxidant due to its free radical. The ethanolic DPPH solution is violet, and its maximum absorption wavelength is 517 nm.

The colour changes upon neutralization of this free radical from violet to pale yellow by daylight. The decolouration of the initial colour is proportional to the test substances having anti-radicalizing power. The absorbance of different concentrations (12.5, 25, 50, 100, 200 and 400 µg/ mL) of tested sample was measured at 517 nm by using UV-7504 spectrophotometer. It was found that as the concentrations were increased, the absorbance values decreased. The percent radical scavenging activity of crude extracts and standard BHT are tabulated in Table 4. The greater the % RSA, the greater the antioxidant activity. In contrast, the lower the IC_{50} indicates the more effective antioxidant activity. From the experimental results, flowers of Sagawa were found to have antioxidant activity. IC_{50} values of ethanol and watery extracts are 26.408 and 51.755 µg/mL, respectively.

According to the result, the ethanol extract was found to be more antioxidant potency than watery extract. The antioxidant potency of ethanol and watery extracts was concluded to be weak when compared with the potency of standard butylated hydroxytoluene (IC_{50} = 22.51 µg/mL). The

IC_{50} values of ethanol and water crude extracts of Sagawa flowers and standard butylated hydroxytoluene are shown in Table 5 and Figure 4.

The scavenging capacity against DPPH used for determining antioxidant activity has been proven to exhibit a positive linear correlation with phenolic compounds and flavonoid compounds, stating that these compounds contribute to the antioxidant capacity of ethanol and watery extracts of Sagawa flower. The results revealed a highly significant correlation between antioxidant activity and total phenolic and total flavonoids contents.

Table 4 Radical Scavenging Activity of Watery and Ethanol Extracts from *M. champaca* (Sagawa) Flower and Standard BHT

Samples	% RSA±SD of different concentrations (µg/mL)					
	12.5	**25**	**50**	**100**	**200**	**400**
Watery extract	11.35 ± 0.005	39.30 ± 0.035	48.68 ± 0.006	86.02 ± 0.011	91.04 ± 0.002	100.65 ± 0.016
Ethanol extract	21.12 ± 0.009	49.39 ± 0.014	60.18 ± 0.002	73.40 ± 0.002	88.90 ± 0.0007	93.01 ± 0.018
BHT (Std.)	34.36 ± 0.004	53.86 ± 0.002	62.38 ± 0.003	66.87 ± 0.004	72.75 ± 0.001	80.65 ± 0.005

Table 5 IC_{50} Values of Crude Extracts from *M. champaca* (Sagawa) Flower and Standard BHT

Samples	**IC_{50} (µg/mL)**
Watery extract	51.76
Ethanol extract	26.41
BHT (Std.)	22.51

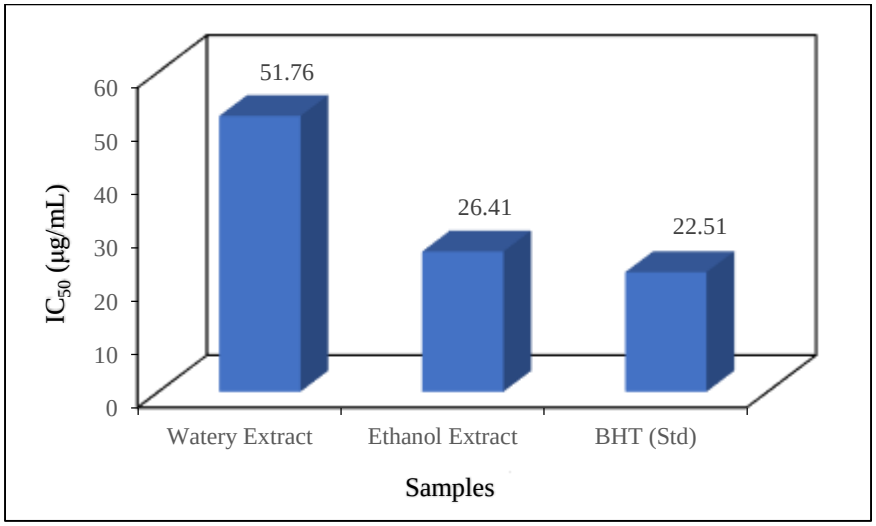

Figure 4 IC_{50} values of watery and ethanol extracts from *M. champaca* (Sagawa) flower and standard BHT

Conclusion

The present study revealed that the extraction yield of watery extract (11.49 %) showed the highest value, followed by the yield of ethanol extract (6.81 %), ethyl acetate extract (2.08 %) and petroleum ether extract (0.78 %). Among the crude extracts, yield percent of watery extract found to be the highest and petroleum ether soluble matter was the lowest in the selected sample. Therefore, *M. champaca* was more soluble in polar solvent. Total phenolic content in ethanol extract (508.95 µg GAE/mg) is higher than that in the watery extract (328.55 µg GAE/mg). In this study, high phenol contents have been found to exert high flavonoids content. The total flavonoids content of watery and ethanol extracts of Sagawa flower was detected to be higher in ethanol extract (9.63 mg QE/g) than watery extract (0.88 mg QE/g). This means that flavonoid compounds were more soluble in ethanol. The antioxidant potential of ethanol extract (IC_{50} = 26.41 µg/mL) is more potent than the watery extract (IC_{50} = 51.76 µg/mL), but weaker activity than standard butylated hydroxytoluene (BHT) (IC_{50} = 22.51 µg/mL). These results might suggest a higher medicinal suitability of alcoholic extracts in various antioxidant applications. The current investigation confirmed that Sagawa flower can be considered as a potential natural source of bioactive phytochemicals such as phenolic compounds and flavonoids that play a major role in human health as free radical scavenger. The ethanolic extract which exhibited the strong antioxidant activity contained the high level of phenolic compounds as well as flavonoids. The results showed significant activities for both phenolic content and antioxidant potential of Sagawa flower. Thus, the natural bioactive compounds from Sagawa flower can be used as the synthetic antioxidant in food, pharmaceutical and cosmetic products.

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