Potent Antioxidant Activity of *Cassia siamea* Flowers and Adsorption Capacity of Dried Leaves of *Cassia siamea* Lam.

Win Win Khaing[1], ThidaTin[2], San San Win[3]

Abstract

This study is an attempt to determine antioxidant activity of the flowers and to investigate the adsorption capacity of dried leaves of *Cassia siamea* Lam. The antioxidant activity of the flowers was determined by Radical Scavenging Assay. The elemental compositions were determined by Energy Dispersive X-ray Fluorescence (EDXRF) and the surface morphology of the dried leaves was examined by using Scanning Electron Microscopy (SEM) at different magnification. The adsorptive property of the dried leaves on heavy metal, lead was determined by complexometric titration. The removal of lead II ions from aqueous solution was carried out by the use of the dried leaves at various concentrations of lead II nitrate solution, pH values ranging from 3 to 6, different contact time between adsorbent and adsorbate and different adsorbent dosage. According to the experimental results, the flowers have potent antioxidant activity. The dried leaves are effective adsorbent for removal of lead in aqueous solution. Optimum pH for removal of lead was 5. The more amount of adsorbent was used, the greater removal of lead was. Similarly, the longer the contact time between adsorbent and adsorbate, the stronger adsorption capacity was.

Keywords: Adsorbent, *Cassia siamea*, EDXRF, Radical Scavenging Assay, SEM

Introduction

It is well known that the Union of Myanmar is a country with a rich source of traditional indigenous medicinal plants. The plants contain many chemical compounds. Due to this chemical richness, different parts of different plants are used in different herbal medicines for various purposes. Furthermore, these are also considered as adsorbents to remove toxic materials in water. This research is mainly concerned with the flowers and dried leaves botanically called *Cassia siamea* Lam. (Ah Shin Naganthein, 1976). The dried leaves were used as adsorbent to reduce lead metal from aqueous solution and the antioxidant activity of the flowers was evaluated.

The substance that is removed from the liquid/gas phase is called the *adsorbate* and the solid surface that is used to remove adsorbate is called *adsorbent.* This phenomenon is called *adsorption* .The phenomenon of adsorption is essentially an attraction of adsorbate molecules to an adsorbent surface. Adsorption processes can be classified as either physical adsorption (van der Waals adsorption) or chemisorption (activated adsorption) depending on the type of forces between the adsorbate and the adsorbent. Physical adsorption is a process in which the electronic structure of the atom or molecule is barely perturbed upon adsorption. Physical adsorption takes place with formation of multilayer of adsorbate on adsorbent. Chemisorption is a kind of adsorption which involves a chemical reaction between the surface and the adsorbate. New chemical bonds are generated at the adsorbent surface (Gawande, *et al*., 2016).

The botanical description of the *Cassia siamea* Lam. was described as the following.

1
2
3

Botanical Description

Botanical Name	: *Cassia siamea* Lam.
Family	: Caesalpiniaceae
Myanmar name	: Mezali
Common name	: kassod tree
Part Uses	: Flowers and dried leaves

Figure 1 Plant, Flowers, Dried Leaves *Cassia siamea* Lam.

Materials and Methods

Sample collection and preparation

The flowers of *Cassia siamea* Lam. were collected from Mandalay University Campus, Mandalay Region on November, 2018 and the dried leaves were collected from the same collecting area on March, 2018. The sample was cut into small pieces and allowed to dry in air. The air dried sample was made to powder and was used throughout the experiment.

The dried powder (500 g) was percolated with 95% ethanol (1500 mL) for two weeks and filtered. This procedure was repeated for three times. The combined filtrates were evaporated. Consequently, 95% ethanol soluble extract was obtained. Water extract of this sample was prepared by boiling 500 g sample with 1000 mL of distilled water for 6 hours and filtered. It was repeated three times and the filtrates were combined followed by heating on water bath and sand bath to obtain water extract. Each extract was stored in refrigerator for screening of antioxidant activities.

Preliminary phytochemical investigation of the flowers of *Cassia siamea* Lam.

All phytochemical tests of the sample were carried out based upon J. B. Harborne (1973), phytochemical methods (London: Chapman and Hall) and New Journal of Science by Chuwuma S. Ezeonu and Chigozie M.Ejikeme, 2016. But in this research, 2g of sample was used as the starting weight for all experiments.

Screening of antioxidant activity of the flowers of *Cassia siamea* lam. by DPPH radical scavenging assay

Preparation of DPPH solution

DPPH (60M) solution was freshly prepared in the pure reagent bottle. The stock solutions of ethanol and watery extracts were prepared by adding 20 mg of each tested sample and 100 mL of ethanol thoroughly by using shaker. The sample solutions of each extract with different concentration of 20,10,5, 2.5, 1.25 and 0.625 g/mL were prepared by serial dilution with ethanol from the stock solution.

Determination of antioxidant activity

The DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity was determined by UV- visible spectrophotometric method according to the procedure described by (Marinova and Batchvarov). The control solution was prepared by adding ethanol (1.5 mL) and 60 M DPPH (1.5 mL). The test sample solutions were also prepared by mixing 1.5 mL of 60 M DPPH solution and 1.5 mL of each sample solution. These mixture solutions were incubated at room temperature and were shaken on shaker for 30 minutes. Ascorbic acid was used as standard. The standard solution was two-fold serially diluted with ethanol to get the standard solutions with the concentration of 20,10,5, 2.5, 1.25 and 0.625 g/mL. Then the absorbance of these solutions was measured at 517 nm by using UV spectrophotometer. The IC_{50} (50% inhibitory concentration) values were calculated by linear regressive excel program from plot of % radical scavenging activity against concentrations (Manjiusha, *et al*, 2014).

Analysis of the flowers and dried leaves of *Cassia siamea* lam. by EDXRF method

The elemental compositions of the selected samples were analyzed by using Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer.

Removing colour of the leaves powder by using 2% sodium hypochlorite

The colour of the leaves powder was removed by 2% sodium hypochlorite solution not to interfere the end point of the processing titration due to its colour.

Characterization of dried leaves powder by scanning electron microscope

It is necessary to characterize the surface morphology of the materials which will be used as adsorbents. Therefore the leaves powder was examined by scanning electron microscope (SEM) for a visual inspection of external porosity and morphology.

Determination of adsorption capacity of dried leaves powder by shaking process

The adsorption capacity of dried leaves powder was determined by shaking process based upon various pH and various concentrations of aqueous lead II nitrate solution, various dosage of the leaves powder (Singh, 2017).

Determination of removal of lead II ion by dried leaves powder at various pH values of lead II nitrate solution

Each 1 g of dried leaves powder sample was mixed 100 mL of 0.01 M lead (II) nitrate solution at pH 3, 4, 5 and 6. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample. The resulting filtrate (10 mL) was placed in a conical flask. 0.2 g of tartaric acid was added. And then 5 mL of buffer solution and 35 mL of distilled water was added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution was changed from colourless to purple. Finally, this solution was titrated with 0.01 M EDTA solution. At the end point, the colour of solution changed to blue.

Determination of removal of lead II ion by dried leaves powder at various concentration of lead II nitrate solution

Each 1 g of dried leaves powder sample was mixed with 100 mL of each lead (II) nitrate solution in the range of 0.006 M, 0.008 M, 0.01 M, 0.012 M and 0.014 M at pH 5. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties. The filtrate (10 mL) was placed in a conical flask and 0.2 g of

tartaric acid was added. Then 5 mL of buffer solution and 35 mL of distilled water was also added into the flask. After adding 0.2 g of EBT indicator, the colour of the solution changed from colourless to purple. The filtrate (10 mL) was placed in a conical flask and 0.2 g of tartaric acid was added. Then 5 mL of buffer solution and 35 mL of distilled water were also added into the flask. After adding 0.2 g of EBT indicator, the colour of the solution changed from colourless to purple. The purple coloured solution was titrated with 0.01 M EDTA solution. When the colour of solution changed to blue, the titration end point was achieved. (Webb,P.A., 2003).

Determination of removal of lead II ion by dried leaves powder at various shaking time

Each 1 g of sample powder was mixed with 100 mL of 0.01 M lead (II) nitrate solution at pH 5. The mixture was shaken for 2 hours, 4 hours, 6 hours, 8 hours and 10 hours by shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample. The filtrate (10 mL) was placed in a conical flask. 0.2 g of tartaric acid was added. And then, 5 mL of buffer solution and 35 mL of distilled water were added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution changed from colourless to purple. Finally, this colour solution was titrated with 0.01 M EDTA solution. At the end point the colour of the solution changed to blue.

Determination of removal of lead II ion by various dosage of sample

In the dosage method, a constant concentration of metal ion (initial concentration of metal ion solution 0.01 M) was used. The dosage of the adsorbent was varied in the range of 0.5 g, 1.0 g, 1.5 g, 2.0 g and 2.5 g. The pH was adjusted to the optimal pH 5 of metal ion. The mixture was shaken for two hours by using shaker at 250 rpm. After shaking, it was filtered and the filtrate was used to determine the adsorptive properties of sample. The filtrate (10 mL) was placed in a conical flask and 0.2 g of tartaric acid was added. And then 5 mL of buffer solution and 53 mL of distilled water were added into the flask. When 0.2 g of EBT indicator was added, the colour of the solution changed from colourless to purple. When this purple colour solution was titrated with 0.01 M EDTA solution, the colour of solution changed to blue at the end point.

Results and Discussion

Phytochemical constituents of the flowers of *Cassia siamea* Lam.

From the results of phytochemical screening, it was observed that the tested flowers contained various phytochemicals including flavonoid, alkaloid, polyphenol and tannins. Especially the presence of flavonoid compound in the flowers is the most interesting factor for its antioxidant activity.

Antioxidant activity of the crude extracts of the flower sample

The antioxidant activity of watery and ethanol extracts the flower of *Cassia siamea* Lim. were evaluated by DPPH (1,1-diphenyl-2-picryl-1hydrazyl) radical scavenging assay (Marinova, *et al*., 2011). The ascorbic acid was used as standard (Md.Nur Alam *et al.*, 2012). From this investigation, it shows that % inhibition increased with increasing the concentrations of crude extracts. Ethanol extract has more potent antioxidant activity than watery extract. However, antioxidant potencies of these extracts were not as good as standard ascorbic acid. The resultant % inhibitions for different concentrations of the crude extracts are shown in Table 1.

Table 1 Percent inhabitation and IC_{50} values of the flower crude extracts

Sample extract	% radical scavenging assay, (mean ± SD) in different concentration(g,mL)						IC_{50}(g/mL)
	0.625	1.25	2.5	5	10	20	
Ethanol extract	13.50 ± 0.02	20.80 ±0.05	29.70 ±0.21	30.20 ±0.25	52.00 ±0.99	59.52 ±0.65	7.2
Watery extract	10.30 ±0.10	14,15 ± 0.98	15.85 ± 0.85	30.98 ± 1.20	45.67 ± 1.25	60.55 ± 0.35	12.5
Ascorbic acid	25.10 ±1.1	53.22 ± 0.75	65.45 ±0.98	73.98 ±0.89	803.98 ± 0.88	92.30 ±0.25	1.15

Elemental compositions of the two tested samples by EDXRF method

Table 2 The Elemental compositions of the flowers and dried leaves by EDXRF

No	Element	Flowers Relative abundance (%)	Dried leaves Relative abundance (%)
1	Potassium (K)	0.970	1.306
2	Calcium (Ca)	3.197	2.980
3	Chlorine (Cl)	4.325	0.262
4	Silicon (Si)	1.059	0.190
5	Aluminium (Al)	0.052	0.099
6	Phosphorous(P)	0.229	0.228
7	Sulphur (S)	0.050	0.082
8	Iron (Fe)	0.035	0.046
9	Strontium (Sr)	0.016	0.026
10	Manganese (Mn)	0.009	0.007
11	Titanium (Ti)	0.009	0.005
12	Barium (Ba)	0.004	0.005
13	Zinc (Zn)	0.005	0.003

In the above operation, relative abudance of the elements present in the tested samples were determined by EDXRF spectrometer. X-ray spectroscopy permits simultaneous analysis of light elements to heavy elements. The major elements and trace elements which are present in these samples are shown in table 2. From the EDXRF report, it can be recorded that the tested samples are rich sources of minerals for health benefit especially potassium and calcium. Meanwhile, it is also known that the toxic metals such as lead, mercury are not present in these samples.

SEM image studies

The surface morphology of the dried leaves powder was examined by scanning electron microscope. Micrographic images were shown in figure 2. It can be observed that the range of particle size in *Cassia siamea* Lam.(Mezali) is 3.15 m to 5.26 m and the shape of the particle is spherical. This is the most interesting factor to use as the adsorbent.

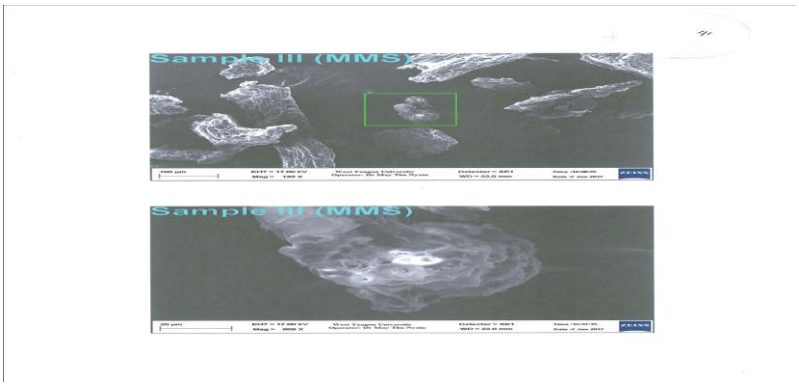

Figure 2 electron micrographic image of dried leaves *of Cassia siamea* Lam.

Effect of pH on the removal of lead II ions

The value of pH is greatly affected on the adsorption of heavy metals especially on lead based upon the adsorbent. As shown in table 3, it was observed that the optimum pH for removal of lead ion in aqueous solution was found to be at pH 5.

Table 3 Effect of pH on the removal of lead II ions

No	pH	Initial conc: mg/L	Final conc: mg/L	Reduced conc: mg/L	Reduced Percentage
1	3	2072.1	1616.24	455.86	21.99%
2	4	2072.1	1574.79	497.31	24.00%
3	5	2072.1	1512.63	559.37	26.99%
4	6	2072.1	1636.96	435.14	20.99%

Table 4 Effect of concentration on the removal of lead II ion at pH-5

No	Lead II nitrate concentration	Initial conc: mg/L	Final conc: mg/L	Reduce conc: mg/L	Reduced Percentage
1	0.006 M	2486.52	1719.84	766.68	30.83%
2	0.008 M	2279.31	1616.24	663.07	29.09%
3	0.01 M	2072.1	1512.63	559.47	27%
4	0.012M	1864.89	1388.31	476.58	25.55%
5	0.014 M	1657.68	1263.98	393.7	23.75%

From the observation of concentration effect of lead, it was noticed that the greater the concentration of lead, the lesser the removal. These results are described in 4.

Effect of contact time on the removal of lead II ion

As shown in the following table, the longer the contact time between adsorbent and adsorbate, the higher the percentage removal of lead but the optimum contact time for removal of lead is 8 hours. Furthermore, it was noticed that the contact time 2 hours can deduce 35% of lead II ion. The resultant data are described in the following table 5.

Table 5 Effect of contact time on the removal of lead II ion at pH-5

No	Contact Time	Initial conc: mg/L	Final conc: mg/L	Reduced conc: mg/L	Reduced Percentage
1	2 hr	2072.1	1346.86	725.24	35%
2	4 hr	2072.1	1305.42	766.68	37%
3	6 hr	2072.1	1243.26	828.84	40%
4	8 hr	2072.1	1181.09	891.01	43%
5	10 hr	2072.1	1118.93	953.17	43%

Effect of Dosage of the sample on the removal of lead II ion

The amount of adsorbent used is greatly affected on the adsorption. In this work, the greater the dosage of the adsorbent, the dried leaves powder, the more the removal percent of adsorbate, lead, in aqueous solution. Moreover it was found that even 0.5 g sample powder can reduce 14% lead content in aqueous solution. The observed data are recorded in table 6.

Table 6 Effect of dosage of adsorbent on the removal of lead II ion

No	Adsorbent (g)	Initial conc: mg/L	Final conc: mg/L	Reduced conc: mg/L	Reduced percentage
1	0.5	2072.1	1782.01	290.09	14%
2	1.0	2072.1	1512.63	559.47	27%
3	1.5	2072.1	1305.42	766.68	37%
4	2.0	2072.1	1098.21	973.89	47%
5	2.5	2072.1	891.00	1181.1	57%

Conclusion

From the whole experimental data, it can be concluded that *Cassia siamea* flowers possess not only potent antioxidant activity but also rich in phytochemicals which support the health benefit. Moreover these flowers contain key minerals for health such as calcium and potassium and also trace minerals. The dried leaves powder is an effective adsorbent for removal of toxic lead metal ion in aqueous solution. The extent of removal of lead II ion from aqueous solution depends on pH, the mass of adsorbent used, the concentration of lead II ion in aqueous solution and the contact time between adsorbent and adsorbate. The optimum pH for the removal of lead II ion was observed at pH 5. And then it could be known that the greater the

dosage of adsorbent, the greater the removal of lead II ion. Similarly, the longer the contact time between adsorbent and adsorbate, the stronger the adsorption capacity. Therefore, the present research indicates that biological resources can be used as adsorbents to remove toxic element such as lead from waste water. Moreover, further pharmaceutical investigation on the flowers of *Cassia siamea* Lim. (Mezali) should be carried out.

Acknowledgements

We wish to express our gratitude to responsible persons of 3nd Myanmar-Korea Conference on Useful Plants at Dagon University , Yangon, Myanmar. We also wish to express our gratitude to Rector Dr Aung Naing Soe and Pro-rector Dr Thida, Mandalay University of Distance Education for their interest on our research work .We are extremely grateful to Professor and Head, Dr Aye Aye Myint ,Professor Dr Moe Moe, Department of Chemistry, Mandalay University of Distance Education for their encouragement to submit this paper.

References

Ah Shin Naganthein, 1976. "Pon Pya Say Abidan". III. 3rd Edition, Mingalar printing, press, Rangoon

Chukwuma S. Ezeonu, *et al*, (2016),"Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods". *New Journal of Science, Volume 2016, Article ID 5601327, 9 pages*.

Gawande, N.J., Chaudhari, A.R. and Khope, R.U., (2012), "Influence of Surface Characteristics of Adsorbent and Adsorbate on Competitive Adsorption Equilibrium", *Advances in Applies Science Research*, Peligia Research Library, ISSN: 0796-8610, 3(3): 1836-1841

Harborne J.B, 1982. "Phytochemical Methods", A Guide to Modern Technique of Plant Analysis, "Chapman and Hall Ltd, USA".

Manjiusha Choudhary, *et al*, (2014)," Phytochemical and Pharmacological activity of Genus Plumeria: An updated review". *International Journal of Biomedical And Advance Research.ISSN: 2229-3809.*

Marinova, G. and V. Batchvarov, (2011) Evaluation of the Methods for Determination of the Free Radical Scavenging Activity by DPPH,Bulgarian Journal of Agricultural Science, vol. 17, p. 1124.

Md.Nur Alam*, *et al*, (2012)," Review on in vivo and in vitro methods evaluation of antioxidant activity". *Saudi Pharmacrutical Journal (2013) 21,143-152*.

Monika Gupta, *et al*,(2016),"Phytochemical screening of Leaves of Plumeria alba and Plumeria acuminate*". Journal of Chemical and Pharmaceutical Research,2016,8(5):354-358.*

Singh, A.K., Srivastava, M., Rajneesh, N.K. and Shukla, S., (2017), "Corncobs as Low Cost Bio-Adsorbent for Water and Waste Water Treatment", *Internal Journal of Innovative Research in Science, Engineering and Technology*, ISSn: 2347-6710, DOI:10.15680.

Webb,P.A., (2003), "Introduction to Chemical Adsorption Analytical Techniques and their Applications to Catalysis", *MIC Technical Publications*.