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Natural Dye from *Curcuma Longa* L. and Its Cytotoxicity and Antibacterial Activities

Myat Lay Nwe[a]*, Amar Khin[b], Win Win Khaing[c]

[a]*Associate Professor, Engineering Chemistry, Technological University (Kyaing Tong), Kyaing Tong, Myanmar*

[b]*Lecturer, Chemistry, Defence Services Technological Academy, Maymyo, Myanmar*

[c]*Associate Professor, Chemistry, University of Distance Education, Myanmar*

[a]*Email: mlnwe2008.mn@gmail.com*

[b]*Email: amrkhinchem@gmail.com*

Abstract

In the present study, *Curcuma longa* L. was selected to extract natural dye. The selected plant material was analyzed for its phytochemical constituents, physiochemical properties, antibacterial properties and its cytotoxicity. For phytochemical test and dye extractions, three solvent systems, ethanol only, 50% ethanol and water were used. Phytochemical test performed on ethanol extract showed that they contained all tested secondary metabolites. The EDXRF results of dye powder expresses it does not contain toxic heavy metal and contains lower mineral contents. The physicochemical property of dye powder expresses it was suitable for dye processes. Cytotoxicity determination was carried out by using the brine shrimp lethality bioassay method. The LC_{50} values of the extracts were determined by linear regression analysis method. It was observed that the ethanol soluble dye powder LC_{50} value was 323.752 ppm, which was more potent compared to water soluble potion with LC_{50} value of 207.182 ppm. The lower concentration of dye powder is suitable for food dye as well as textile dye.

Keywords: natural dye; *Curcuma longa* L; cytotoxicity; antibacterial properties; food dye.

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* Corresponding author.

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1. Introduction

Nowadays natural dye is very popular for food dye as well as food preservative. Many foodborne illnesses can cause when the food contaminant with the spoilage, pathogenic bacteria, viruses, or parasites. Natural antimicrobial food preservatives or bio-preservation can safe these problem [1]. Bio-preservation was used as natural preservatives against and those preservatives are obtained from animals, plants, bacteria, as well as mushrooms, algae, and viruses [2]. The demand for natural antimicrobial agents is expected to increase steadily for replacing synthetic compounds [3]. The colour of a food substance indicates freshness and safety that are indices of good aesthetic and sensorial values [4]. Synthetic colours are widely used in the food, pharmaceutical, textile and chemical industries. Although synthetic food dyes have several important traits in economic point of view such as low cost, resistance to light, oxygen, and pH changes, and high colour stability, synthetic dyes have led to severe health problems such as cancers, allergic reactions, hyperactive effects on children, irritability, effects on the liver, kidney, and intestine, and asthma [5]. The food processing is rising year by year and the use of food additives including food dyes also increasing. Synthetic food dyes are less expensive and have intense than natural food dyes but have general toxicity [6]. There are several research works on the application of natural dyes have been reported to protect the environment pollution [7]. There are many natural dye resources; Myanmar people has been used turmeric for various purposes and through different routes on food dye and also used as traditional medicine to treat the skin for wounds, blistering diseases such as pemphigus and for parasitic skin infections, and for acne. Turmeric powder has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a blood purifier in Myanmar. Turmeric (*Curcuma longa*) rhizome is a member of the ginger family Zingiberaceae [8]. The rhizomes of turmeric provide a yellow, flavourful powder when dried and ground and have long been used in Chinese, Indian traditional medicines [9]. Turmeric has also attracted considerable attention over the years due to its use in the food industry as a coloring agent [10]. Turmeric and its extract have various beneficial effects on human health [11]. Turmeric owes its characteristic yellow color to three major pigments Cumarrin and its derivatives [12]. Curcumin is an important natural colorant used in food, which has a wide range of pharmacological activities [13]. It has anti-microbial effects against many microorganisms, especially against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus* [14, 15]. In this research work the natural yellow dyes were extracted from rhizome of turmeric and it antibacterial activities and cytotoxic were determined.

2. Material and Methods

The chemicals used in my research work were purchased from British Drug House (BDH), England and Wako Chemical Co. Inc. Tokyo, Japan. Analar grade reagents and solvents were used thought the experiment. The recommended standard methods and techniques involved both conventional and modern methods were used in all the investigations.

2.1. Sampling

In this research work, turmeric's rhizome was collected from the Kyaukse Township, Mandalay Region. The collected rhizome was washed with tap water to remove unwanted material adhered on the surface. Air dried

samples were cut into small pieces and then stored in brown glass bottles in dried and dark place till further used. Phytochemical tests for the sample were determined according to the standard methods as described by Harbone (1973) [16].

2.2. Examination of suitable solvent

Dye were extracted from the natural source, the rhizome of turmeric by using three solvent systems such as EtOH only, hydroethanol (1:1 v/v) and water only. The visible color changing intensities were measured by using UV spectrophotometer

2.3. Extraction of natural dye

Each 10 g dried samples, turmeric was placed into the 2.5 L bottle and it was filled with 100 mL of EtOH only. The bottle was placed into a water bath and temperature was maintained at 70ºC for 60 min. After one hour, the dye solution was cooled and filtered. The solvent was evaporated by using rotary evaporator. The dye powder percent was calculated based on the dried rhizome weight. Some physical parameters were measured.

2.4. Physicochemical properties analysis

Natural dye extracted from turmeric rhizome was subjected to its physicochemical characteristics such as appearance, moisture, melting point, solubility in cold water, hot water and methanol, pH of 1 % solution, total ash, water soluble ash and acid insoluble ash using standard methods. All the physic-chemical determinations were triplicated. Mineral content of dye powder was determined by EDXRF, at Chemistry Department of Monywa University. The moisture content of dye powder was determined oven dried method. The melting point of dye powder was determined at Chemistry Department, Yadanabon University.

2.4.1. Determination of color appearance

The dye powders were placed separately on glass plates and observed carefully for their general colour appearance. The observation was recorded as accurately as possible replicated three times.

2.4.2. Determination of moisture content

Moisture content of dye powder was determined by standard gravimetric method. 2 g of dye powder was dried in an oven at 100°C ± 5°C initially for 2 hours and weight. And then it was dried again and weight. Theses procedure was done repeatedly till constant weight was obtained.

2.4.3. Determination of solubility

Each dye powder (0.1g) was placed into the 100 mL beaker and 10 mL of cold water, hot water and methanol, respectively. These dye solutions were stirred with glass rod and these dye solutions were allowed to stand for 2 minute and filtered. The residues were dried and examined their weight.

2.4.4. Determination of total ash

Total ash was determined with the method of the Pharmacopoeia of India. The dye powder was placed in a crucible with lid. The closed crucible was ignited on the stove. The crucible was ignited until free from carbon. Percentage of ash was calculated with reference to the amount of dye initially taken.

2.4.5. Determination of water insoluble ash

Water soluble ash content was determined according to the Pharmacopoeia of India. The ash was boiled with 25 mL of water for five minutes and the insoluble matter was collected in a pre-weighed crucible and heated till constant weight. The water soluble ash was calculated with reference to the initial quantity of dye.

2.4.6. Determination of acid insoluble ash

Acid insoluble ash content was determined according to the Pharmacopoeia of India. The ash was boiled with 25 mL of 0.05 M hydrochloric acid for five minutes and the insoluble matter was collected in a pre-weighed crucible and heated till constant weight. The water soluble ash was calculated with reference to the initial quantity of dye.

2.5. Determination of antibacterial activity

Antibacterial activity of dye powder was determined at Biotechnology Department, Mandalay Technological University. Dye powder extracts were prepared such as watery extract (C2-a) and ethanol (C2-b). The screening of antimicrobial activities of each watery and ethanol extracts on the tested bacteria was determined by the using agar well diffusion method because of its simplicity, speed of performance and economy. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. Each 20µl of extracts was inoculated onto wells were made in the spread plate culture of each microbial isolates. All plate of the tested organisms was incubated at 35$\pm$2°C for overnight. The diameters of the inhibition zones were measured by measuring scale in millimeter (mm).

2.6. Brine Shrimp lethality assay

In Cyto-toxicity bioassay, firstly the brine shrimp eggs were hatched. Three tea spoons brine shrimp eggs were placed in conical shaped vessel (one liter) through a vial containing sterile artificial seawater. It was controlled for under aeration 48 hours. Active nauplii used for this assay. After hatching thirteen nauplii were drawn through glass capillary tubes and then placed in a vial containing 4.5 ml of brine solution and 0.5 ml of sample solution. It was maintained at room temperature for 24 hours under light. Beyond 48 hours, surviving larvae were counted. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} value was obtained from the best-fit line plotted concentration verses percentage lethality. Potassium dichromate was used as a positive control in the bioassay. The mean results of mortality percentage of the brine shrimp versus the log of concentrations were plotted using the Microsoft Excel spreadsheet application, which also formulated the regression equations. These equations were later used to calculate LC_{50}

values for the samples tested with consideration of value greater than 1.0 mg/mL, suggesting that the extract is nontoxic.

3. Results and Discussion

3.1. Phytochemical screening

The phytochemical screening results reveal that the presence of several groups of secondary metabolites in rhizome of turmeric. All tested phytochemical constituents are present in ethanol extract. Tannin, steroid and terpene are not found in watery extract. In 50% ethanol, all tested constituents are found except tannin. The dye was extracted with three different solvents, EtOH, 50% EtOH and watery. The color intensities of dye solutions were checked by UV spectrophotometer. 50% EtOH and watery extracts give high intensity and peak patterns are too broad and wide (600-380 nm). EtOH extract gives narrow peak between (530-400 nm) and the band pattern be incisively seen. Therefore EtOH solvent was chosen for dye extraction solvent.

Table 1: Phytochemical composition of sample

Phytochemical metabolites	Extracts		
	Ethanol	50% Ethanol	Watery
Alkaloid	+	+	+
Flavonoid	+	+	+
Saponin	+	+	+
Glycoside	+	+	+
Tannin	+	-	-
Phenolic	+	+	+
Steroid	+	+	-
Terpene	+	+	-
Polyphenol	+	+	+
Reducing Sugar	+	+	+

+ present ; - absent

The percent yield of dye powder was calculated based on the dried plant materials initially taken. The yield percent of dye powder from and rhizome of turmeric under extraction time (60 min), solid liquid ratio (1:10 w/v), extraction temperature (70 $\pm$5 ºC) optimized conditions was found to be 6.2 %.

3.2. Mineral contents

The dye powder was sent to Chemistry Department, Monywa University to determine mineral content of the sample. According to the EDXRF data, the dye powder contains lower mineral contents. Among containing minerals, silica is the highest amount (1.117 %), and sulphur is the second higher composition (1.033 %) in turmeric. Toxic heavy metals are not found in this sample.

Table 2: Metal content in turmeric powder

Metal	Results (%)
Silica	1.117
Sulphur	1.033
Calcium	0.110
iron	0.005
Manganese	0.002
Chromium	0.001
Molybdenum	0.001

3.3. Physicochemical properties

The natural dye power from tumeric rhizome appeared as yellow powder and its melting point is 140 ± 3 °C. Solubility of dye powder in cold water was 65.4 %; in hot water was 93.4 %; in methanol was 81.55 %, respectively. The percentage total ash, water soluble ash and acid insoluble ash content of dye powder was found to be 3.3, 1.2 and 1.0 respectively. When the dye powder was used in dyeing process, the dye powder was firstly dissolved in hot water and stirred till all dye soluble in hot water. Residue on ignition of dye powder was nearly in the range of 0.93 % which is very low. These observations and values can be considered as characteristic properties of dyes in quality control process. Ash values in powder represent the presence of inorganic matter. High water soluble ash and acid insoluble ash values suggest that the presence of high mineral. The calculated extracted values of the natural dyes from the plants under investigation have not been reported earlier. The values of physicochemical characteristics of the turmeric rhizome dyes may serve as standard and physicochemical markers in authentication.

Table 3: Physiochemical properties of dye powder

No.	**Physicochemical properties**	**Observation value**
1	Color appearance	deep Yellow
2	Moisture (%)	4.2 ± 0.02
3	Melting point (ºC)	140 ± 3
4	Solubility in cold water (%)	65.4 ± 0.1
5	Solubility in hot water (%)	93.4 ± 0.02
6	Solubility in Methanol (%)	81.55 ± 0.02
7	Total ash (%)	3.3 ± 0.04
8	Water insoluble Ash (%)	1.2 ± 0.03
9	Acid insoluble Ash (%)	1.0 ± 0.07
10	Residue on Ignition (%)	0.93 ± 0.01
11	Percent Yield (%)	6.16 ± 0.11

Values are the average of triplicate experiments and represented as mean ± Standard deviation

3.4. Antibacterial properties

The watery extract of dye powder has the lower antibacterial activities on tested six isolated organisms under the study whether gram positive/negative with inhibition zone ranging from (2-7 mm). On the other hand, The ethanol extract of dye powder have the higher antibacterial activities on tested six isolates of the bacteria under the study with inhibition zone ranging from (16-22 mm).

Table 4: Preliminary antibacterial testing of dye extracts through determination of zone of inhibition

Test organisms	Inhibition Zone (mm)		
	Watery extract	**Ethanol extract**	**Control**
Bacillus cereus	2 ± 0.10	18 ± 0.02	-
Staphylococcus aureus	5 ± 0.21	20 ± 0.15	-
Pseudomonas aeruginosa	5 ± 0.03	22 ± 0.05	-
scherichia coli	7 ± 0.01	18 ± 0.11	-
Shigella	5 ± 0.21	20 ± 0.13	-
Salmonella typhii	5 ± 0.11	16 ± 0.13	-

**The diameters of zone of inhibition were expressed in millimeter (mm) as mean ± standard deviation (SD).*

3.5. Cytotoxicity of turmeric dye

Brine shrimp (*Artemia salina* LEACH) is used as a simple bioassay tool for cytotoxicity test on dye extracts. The procedure determines LC_{50} value in μg/ ml of extracts in the brine medium. The advantages of this method are being rapid results, reliability, inexpensive and convenient assay. The LC_{50}value of water soluble potion is 207.182 ppm whereas that of ethanol soluble potion is about 323.752 ppm. Standard brine shrimp lethality bioassay stipulates that an LC_{50} value < 1000 ppm is considered bioactive in toxicity evaluation of plant extracts. Based on this benchmark, the LC_{50} value of watery and ethanol extracts were both < 500 ppm, this implies that they are highly cytotoxic. The phytochemical test result clearly indicates that the EtOH extract contains more secondary metabolites with cytotoxic activity compared to watery extract.

Table 5: Mortality for chronic toxicity of $K_2Cr_2O_7$

Dosage (ppm)	**Log Dosage**	**Alive**	**Dead**	**% Dead**
6.25	0.79	30	0	0
12.5	1.09	30	0	0
25	1.39	25	5	16.66
50	1.69	10	20	66.66
100	2	3	27	90.00

95% confidence limit of LC_{50} = 41.67 ppm

Table 6: Mortality for Chronic Toxicity of water soluble potion (C2 a)

Dosage (ppm)	Log Dosage	Alive	Dead	% Dead
500	2.6989	2	28	93.33
250	2.3979	14	16	53.33
125	2.0969	18	12	40.00
62.5	1.7958	19	11	36.66
31.25	1.4948	20	10	33.33
15.625	1.1938	23	7	23.33
7.8125	0.8927	24	6	20.00
3.9	0.5910	24	6	20.00
1.9	0.2787	25	5	16.66
0.95	-0.0222	30	0	0.00
0.475	-0.3233	30	0	0.00

95% confidence limit of LC_{50} = 207.182 ppm

Table 7: Mortality for Chronic Toxicity of ethanol soluble potion (C2 b)

Dosage (ppm)	Log Dosage	Alive	Dead	% Dead
500	2.6989	9	21	70.00
250	2.3979	19	11	36.66
125	2.0969	20	10	33.33
62.5	1.7958	21	9	30.00
31.25	1.4948	21	9	30.00
15.625	1.1938	23	7	23.33
7.8125	0.8927	24	6	20.00
3.9	0.5910	25	5	16.66
1.9	0.2787	25	5	16.66
0.95	-0.0222	30	0	0.00
0.475	-0.3233	30	0	0.00

95% confidence limit of LC_{50} = 323.752 ppm

4. Conclusion

In this research, the natural dyes were extracted from *Curcuma longa* L., rhizome of turmeric. According to phytochemical screening, turmeric contains all types of tested secondary metabolites contain in EtOH extract. Quantitative elemental analysis by using EDXRF revealed that dye powder extracted by using EtOH solvent has little amount of mineral and does not contain toxic heavy metal. EtOH soluble dye powder has high antibacterial activity. The high dosage of water and ethanol soluble parts have cytotoxic. Home Economics and

as suitable organic replacements for the chemical colorants used in food dye.

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References

[1]. Pisoschi, A.M.; Pop, A.; Georgescu, C.; Turcu¸s, V.; Olah, N.K.; Mathe, E., "An overview of natural antimicrobials role in food". Eur. J. Med. Chem., 143, 922–935, 2018.

[2]. Gyawali, R.; Ibrahim, S.A., "Natural products as antimicrobial agents. Food Control", 46, 412–429, 2014.

[3]. Dorman, H.J.D.; Deans, S.G., "Antimicrobial agents from plants: antibacterial activity of plant volatile oils". J. Appl. Microbiol. 88, 308–316, 2000.

[4]. Pritam, C., Chatterjee, S. and Sen, K. "Biotechnological potential of natural food grade biocolourants. African". J. Biotechnol. 7 : 2972-2985, 2008.

[5]. Goyette, G.H., Connors, C.K., Petti, T.A. and Curtis, L.E. "Effects of artificial colors on hyperkinetic children: a double-blind challenge study". Psychopharmacol. Bull. 14 : 39-40, 1978.

[6]. Carvalho, P.R. "Potencialidade dos conservantesnaturais". Rev. Bras. de Corantes Naturais. 4 : 244-245, 1992.

[7]. Hebeish, A.A., Ali, N.F. and Abd El-Thalouth, J.I. "Green strategy for development of antimicrobial printed textile fabrics". Res. J. Textile. Apperal. 16 : 77-85, 2012.

[8]. O'Hara M., Kiefer D., Farrell K., Kemper K."A review of 12 commonly used medicinal herbs". Archives of Family Medicine. 7(6):523–536, 1998.

[9]. Aggarwal, B.B., Kumar, A., Aggarwal, M.S. and Shishodia, S. "Curcumin derived from turmeric (Curcuma longa): a spice for all seasons. In: Phytopharmaceuticals in Cancer Chemoprevention" edited by H. Press. 349-387, 2005.

[10]. Joshi, P., Jain, S., and Sharma, V. "Turmeric (Curcuma longa) a natural source of edible yellow colour". International Journal of Food Science and Technology, 44, 2402–2406, 2009.

[11]. Aggarwal, B. B., Kumar, A., and Bharti, A. C. "Anticancer potential of curcumin: Preclinical and clinical studies". Anticancer Research, 23, 363–398, 2003.

[12]. Nishiyama, T.; Mae, T.; Kishida, H.; Tsukagawa, M.; Mimaki, Y.; Kuroda, M.; Sashida, Y.; Takahashi, K.; Kawada, T.; Nakagawa, K. and Kitahara M. "Curcuminoids and Sesquiterpenoids in Turmeric (Curcuma longa L.) Suppress an Increase in Blood Glucose Level in Type 2 Diabetic KK-Ay Mice", 2005.

[13]. Khurana, and Ho, C. "High performance liquid chromatographic analysis of curcuminoids and theirphotooxidative decomposition compounds in Curcuma longa L". J. Liquid Chromatogr., 11, 2295-

2304, 1988.

[14]. Sampathu, S. R., Lakshminarayanan, S., Sowbhagya, H. B., Krishnamurthy, N. and Asha, M. R. (2000). "Use of curcumin as a natural yellow colourant in ice cream". In Paper presented at National Seminar on Natural Colouring Agents, Lucknow, India.

[15]. Egan, M. E., Pearson, M., Weiner, S. A., Rajendran, V., Rubin, D. and Glockner, P. J. "Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects". Science, 304, 600-602, 2004.

[16]. Harbone J.B and Baxter. "The Handbook of Natural Flavonoids 2, Willey, London, 196-199, 1999.