ISOLATION OF SOME CHEMICAL CONSTITUENTS AND COMPARATIVE STUDY ON THE CURCUMIN CONTENT IN *CURCUMA LONGA* L. (NA-NWIN) COLLECTED FROM SOME REGIONS OF MYANMAR

Yu Nwe Moe[1], Khin Hnin Mon[2]

Abstract

In this study, *Curcuma longa* L. rhizomes were collected from six regions (Mawlamyine in Mon State, Kawkareik in Kayin State, Taungyi in Southern Shan State, Ann in Rakhine State, Aunglan in Magway Region and Pathein in Ayeyarwaddy Region) of Myanmar. Preliminary phytochemical investigation of *C. longa* revealed the presence of glycoside, carbohydrate, α-amino acid, flavonoid, terpenoid, steroid, saponin, tannin, phenolic compound. Alkaloid and reducing sugar were absent. The powdered samples were extracted with petroleum-ether (60-80 °C) and followed by 95 % ethanol. Percent curcumin and colour value were determined by UV–visible spectrophotometry. The percent curcumins and color values of *C. longa* were found to be 0.37 % and 62 in Mon State, 0.57 % and 96 in Southern Shan State, 2.27 % and 383 in Rakhine State, 2.94 % and 477 in Magway Region, 3.39 % and 570 in Ayeyarwaddy Region, and 3.8 % and 640 in Kayin State. Curcumin (R_f = 0.44, m.pt = 175 ºC) was isolated from ethanol extract of *C. longa* of Kayin State by column chromatography.

Keywords: *Curcuma longa* L., phytochemical, percent curcumin, colour value, curcumin

Introduction

Human diseases have been treated with plants for thousands of years still nowadays, many currently used medicines are derived from natural sources. *C. longa* requires warm and moist conditions. It can be cultivated in most areas of the tropics and subtropics (Ammon, 1991). It is an important herb and is widely used worldwide as medicine, condiment, dye and cosmetic (Jagan and Sakuriah, 2005). This plant is a perennial herb 60 – 100 cm high with a short stem and tufted leaves. Primary rhizomes are ovate, oblong, pyriform, denominated 'bulb' or round turmeric. The secondary rhizomes are more cylindrical and 4–7 cm long and 1– 1.5 cm wide, called 'fingers' (Eigner and Scholz, 1999). It has been used in traditional medicine as a remedy for various diseases including cough, diabeties and hepatic disorders. The content of rhizomes are very variable and depend on the site of cultivation, type of cultivar, moment of harvest method of processing and method of analysis (Ammon, 1991). *C. longa* is a group of phenolic compounds composed mainly of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Paramasivam, 2008). Curcumin is the main chemical compound of *C. longa* and proven for its anti-inflammatory, anti-oxidant, anti-mutagenic, anti-diabetic, anti- bacterial, hepatoprotective, expectorant and anti-cancerous pharamacological activities. Curcumin has shown anti-proliferative effect in multiple cancers such as colon, skin, stomach, soft palate, tongue, sebaceous glands and breast (Gupta, 2010).

1. Demonstrator, Department of Chemistry, Bago University
2. Dr, Associate Professor, Department of Chemistry, Mandalay University of Distance Education

C. longa (Na-nwin) (Figure 1) has been chosen for this research because it has various biological activities and bioactive chemical constituents. In this research work, screening of phytochemical constituents, comparison of percent curcumin and colour value and isolation of chemical constituents from crude extract of *C. longa* rhizome.

plant rhizome powder

Figure 1 The photograph of plant, rhizome and powder of *Curcuma longa* L.

Botanical Aspect of *Curcuma longa* L.

Family	Zingiberaceae
Genus	*Curcuma*
Species	*Curcuma longa*
Syn	*C. domestica* Valeton
Botanical name	*Curcuma longa* L.
Myanmar name	Na-nwin
English name	Turmeric (Chattopadhyay, 2004)

Materials and Methods

Collection of Sample

The rhizome of *C. longa* (Na-nwin) was collected from Aunglan in Magway Region, Pathein in Ayeyarwady Region, Taungyi in Shan State (South), Mawlamyine in Mon State, Kawkareik in Kayin State and Ann in Rakhine State.

Phytochemical Screening

Preliminary phytochemical investigation of *C. longa* tests such as alkaloids, glycosides, carbohydrates, α-amino acids, flavonoids, terpenoids and steroids, saponins, tannins, phenolic compounds and reducing sugars were carried out according to the appropriate reported methods (Harborne, 1993).

Determination of Percent Curcumin and Colour Value by UV-visible Spectroscopic Method

Preparation of authentic curcumin

25 mg of authentic curcumin was put in a 100 mL volumetric flask. It was dissolved and dilute with ethanol. Then 10 mL of above solution was taken in volumetric flask and again volume made up to 100 mL with ethanol. This authentic solution contains 2.5 mg (0.0025 g/L). Authentic curcumin solution was measured at 425 nm (Himesh, 2011).

Preparation of sample curcumin

0.1 g of dried powder sample was added with 25 mL ethanol and reflux for two and half hour. This solution was cooled and filtered the resulting filtrate was put in a 100 mL volumetric flask. It was dissolved and diluted with ethanol (100 mL). Then 10 mL of above solution was taken in a volumetric flask and again volume made up to 100 mL with ethanol. The absorbance was measured at 425 nm by using UV-1650 spectrophotometer (Himesh, 2011). The rhizome samples of six regions were separately prepared and then percent curcumin and colour value were determined. Percent curcumin and colour values were calculated by using following equation:

$$\text{Absorptivity of Curcumin} = A = \frac{a_1}{L \times C}$$

$$\% \text{ Curcumin sample} = \frac{a_1 \times 100}{L \times A \times m}$$

Where, a_1 = absorbance of authentic curcumin at 425 nm

a_2 = absorbance of extract at 425 nm

L = cell length in cm

C = concentration in gL^{-1}

m = mass in g of sample

colour value = $a_2 \times 1000$ (Himesh, 2011)

Isolation of Chemical Constituents from Crude extract of *Curcuma longa* L. (Rhizome)

Dried powdered rhizome sample of *C. longa* (100 g) was extracted with petroleum ether (250 mL), for 3 days at room temperature (3 times). The mixture was filtered with filter paper. The remaining residue was extracted with EtOH (200 mL) for 3 days at room temperature (3 times). This mixture was filtered with filter paper, and then, the resulting filtrate was evaporated in rotatory evaporator to volume reduce and then complete dryness. Resulting crude extract was weighed. Crude extract (0.21 g) was then subjected to column chromatography. The whole extract was dissolved in chloroform and throughly adsorbed on silica gel. The adsorbed material after being dried was transferred to column which was packed with 20 g of gel in chloroform. The column was eluted consecutively with chloroform: methanol (99:1, 600 mL). Collected each fraction was monitored by TLC using chloroform: methanol (99:1) as solvent system. The spot on TLC were visualized by spaying 5 % ferric chloride.

Determination of R_f Value of the Isolated Compound

R_f value of the isolated compound was determined by thin layer chromatogram using GF_{254}, (Merck) percolated silica gel on aluminum plate, as adsorbent and developed with suitable solvent systems. After the plate was dried, the R_f value of isolated compound was measured. Localization of spot was made by viewing directly under UV-254 nm and 365 nm lamp and or visualized by spraying with staining agent.

Determination of Melting Point

Isolated compound was individually introduced into capillary tubes. The tubes were placed on the Gallenkamp melting point apparatus and heating was started. The melting point was recorded when they started to melt.

Identification of the Isolated Compounds

The isolated compound was identified by FT IR and UV-visible spectroscopy.

Study on FT IR spectrum of the isolated compound

The infrared spectral of the isolated compound was recorded and examined whether the respective functional groups were presented or not by using FT IR spectrophotometer (Perkin Elmer Spectrum GX Fourier Transform in Infrared Spectrometer) at Department of Medical Research (DMR) in Yangon.

Study on the isolated compound by UV-Visible spectroscopy

For the identification of isolated compound, the ultraviolet observation spectrum of the isolated compound was recorded on Shimadzu UV-240, UV-visible spectrophotometer at Universities' Research Centre (URC).

Results and Discussion

Phytochemicals Present in *Curcuma longa* L.

Preliminary phytochemical analysis was performed in order to know different types of chemical constituent present in the plant samples of six regions. The types of chemical constituents were found to be identical in all regions. According to the results of preliminary phytochemical examination (Table 1), it indicated the presence of glycoside, carbohydrate, α-amino acid, flavonoid, terpenoid and steroid, phenolic compound, tannin, and saponins. However, alkaloid and reducing sugar were absent.

Table 1 Phytochemicals Present in *Curcuma longa* L.

No	Types of compounds	Extract	Test reagents	Observation	Remark
1	Alkaloid	1 % HCl	Mayer's reagent	no. ppt	-
			Dragendorff's reagent	no. ppt	-
			Wagner's reagent	no. ppt	-
2	Glycoside	H_2O	10 % lead acetate	ppt (white)	+
3	Carbohydrate	H_2O	10 % α-naphthol and conc: H_2SO_4	Redring	+
4	α-amino acid	H_2O	Ninhydrin reagent	Pink	+
5	Flavonoid	70 %EtOH	Mg turning and conc: HCl	Pink color	+
6	Terpenoids and steroid	PE	Acetic anhydride and conc: H_2SO_4	blue	+
7	Phenolic compound	H_2O	Ferric chloride (5 %)	blue	+
8	Tannin	H_2O	2 % NaCl and 1 % Gelatin	ppt (white)	+
9	Saponins	H_2O	Distilled water	frothing	+
10	Reducing Sugars	H_2SO_4 (dil)	NaOH (dil) and Benedict's solution	no ppt	-

(+) present, (-) absent, ppt = precipitate

Percent Curcumin and Colour Value of *C. longa* Rhizome

The percent curcumin and colour value of *C. longa* rhizome were determined by UV-visible spectrophotometry at 427 nm. The increasing order of percent curcumin contents and colour values from different regions are shown in Table 2.

From these data, the percent curcumin and colour value are the highest in *C. longa* rhizome from Kayin State and the lowest in that from Mon State, compared with other regions: Shan State (South), Rakhine State, Magway Region and Ayeyarwady Region.

Table 2 Quantitative Estimation of Curcumins and the Respective Colour Values in *Curcuma longa* L. (Na-nwin) from Some Regions of Myanmar

Sample	% Curcumin	Colour value
M	0.37	62
S	0.57	96
R	2.27	383
MG	2.84	477
A	3.39	570
K	3.80	640

M = the *C. longa* rhizome sample from Mon State
S = the *C. longa* rhizome sample from Shan Statez
R = the *C. longa* rhizome sample from Rakhine State
MG = the *C. longa* rhizome sample from Magway Region
A = the *C. longa* rhizome sample from Ayeyarwady Region
K = the *C. longa* rhizome sample from Kayin State

Identification of the Compound Isolated from the Crude extract

From the silica gel column chromatographic separation of crude (ethanol) extract of the rhizome of *C. longa*, a compound was isolated as orange needles in 0.6248 % of yield based of raw material. The R_f values of compound were found to be 0.44 and 0.34 ($CHCl_3$: MeOH, 99: 1 v/v and PE: EA, 1: 1 v/v) and identified with that of authentic curcumin and both of these compounds also gave the same behaviours on TLC. In addition, the melting point of isolated compound was observed to be 175 ºC and in literature curcumin is 177 °C (Merck Index, 2001). According to iodine vapour test and 5 % ferric chloride test, brown colour was observed, indicating that phenolic OH groups were present in curcumin. All of these physicochemical properties of compound leads to assign it as curcumin. After characterization, the isolated compound was generally classified and then was identified by FT IR and UV-visible spectroscopy.

Identification of the Isolated Compounds by FT IR and UV-Visible Spectroscopy

According to FT IR spectrum and spectral data, the broad bands at 3600 ~ 3317 cm^{-1} were due to –OH stretching vibration of phenolic hydroxyl groups. Aliphatic C–H stretching vibrations of CH_3 and CH_2 groups were found at 2924 and 2854 cm^{-1}, respectively, carbonyl stretching vibration of keto-enol system was observed at 1620 cm^{-1} and C=C stretching vibrations of aromatic ring system were found at 1597 cm^{-1}, 1504cm^{-1} and 1427 cm^{-1}, a sharp and strong signal at about 1273 cm^{-1} due to =CH in plane deformation. Two sharp and strong signal at about 1195 cm^{-1} due to C=O bending. Asymmetric and symmetric stretching vibrations of C–O–C group were found at 1026 cm^{-1}. Out of the plane bending vibration of trans olefinic C–H was assigned at 964 cm^{-1} and that of aromatic C–H were at 864 cm^{-1} and 810 cm^{-1}. Figure 3 represents FT IR spectrum of curcumin. The corresponding spectral data are summarized in Table 3. Ultraviolet spectrum of curcumin measured in EtOH solvent was illustrated in Figure 4. The wavelength of maximum absorption was observed at 427.86 nm, indicating the presence of conjugated double bond and found to be similar with the literature value (422 nm) (*Kasuge *et al*., 1985).

From the observation of physicochemical properties and spectroscopic evidences, the isolated compound was identified as curcumin.

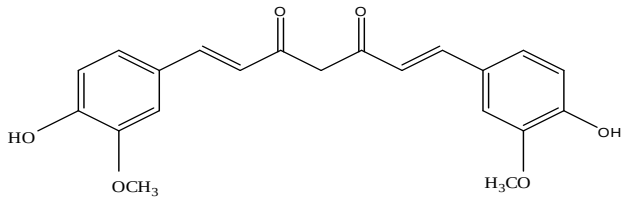

Figure 2 Structure of curcumin

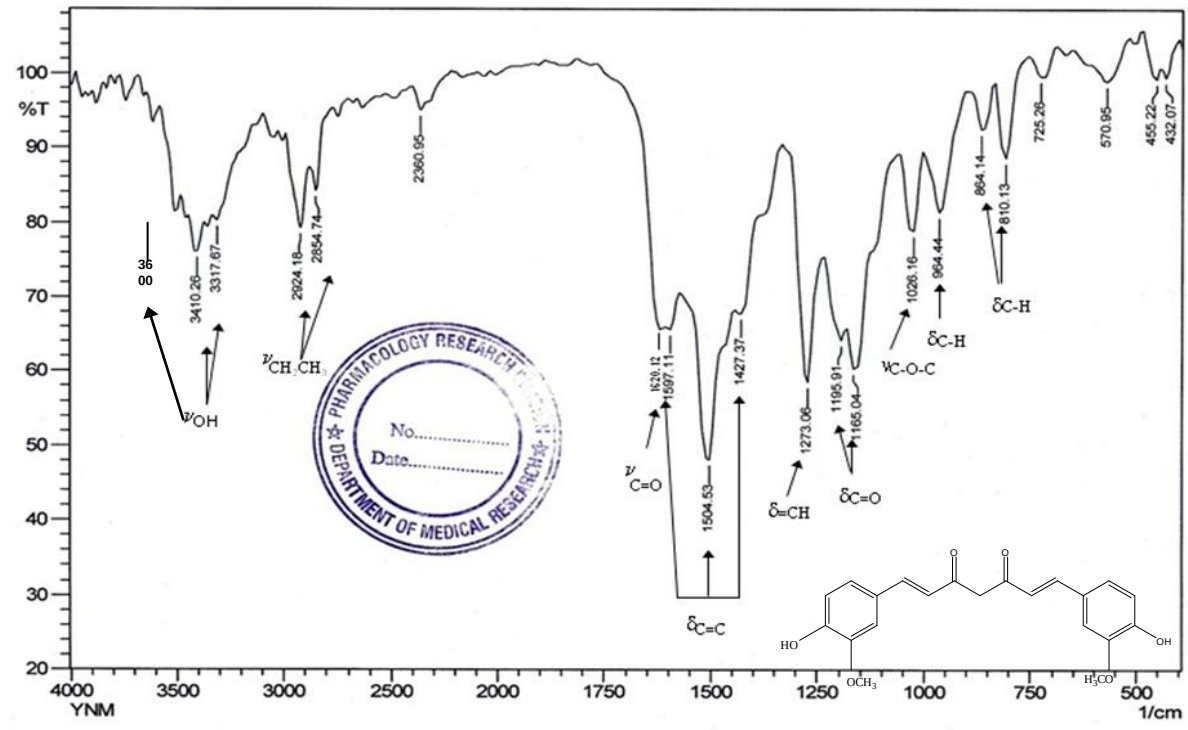

Figure 3 FT IR spectrum of the isolated compound

Table 3 FT IR Spectral Data of the Isolated Compound

Wave number (cm^{-1})		Assignment
Literature*	**Observed**	
3603	3600, 3317	OH stretching of phenolic –OH group
2943, 2827	2924, 2854	Aliphatic C–H stretching of CH_3 and CH_2 group
1628	1620	C=O stretching of keto-enol group
1603	1597	Aromatic C=C ring stretching
1509	1504	
1429	1427	
1283	1273	=CH in plane deformation
-	1195	C=O bending
1026	1026	Asymmetric and symmetric stretching of C–O–C group
962	964	C–H out of plane bending of trans olefinic group
856	864	Aromatic C–H out of plane bending
814	810	

(*Than Soe, 1984)

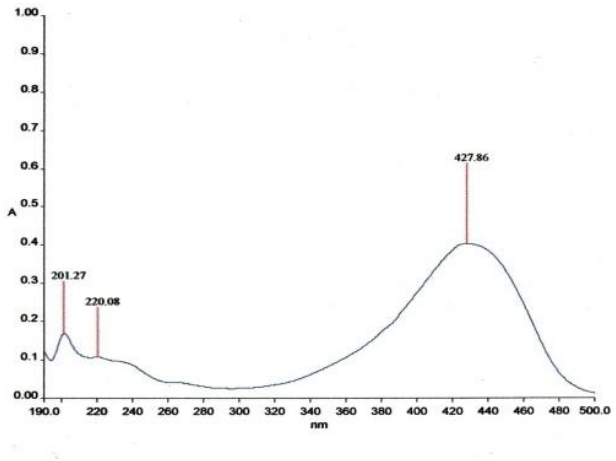

Figure 4 UV spectrum of the isolated compound (ethanol)

Conclusion

From the overall assessment of present work, the following inferences can be deduced.

In preliminary phytochemical investigation, glycoside, carbohydrate, α-amino acid, flavonoid, terpenoid and steroid, saponin, tannin, phenolic compound were present and alkaloid and however reducing sugars were not detected in the sample.

Percent curcumin and colours were determined by UV–visible spectrophotometry from powder samples. The percent curcumins and colour values were found to be 3.8 % and 640 in Kayin State > 3.39 % and 570 in Ayeyarwady Region > 2.94 % and 477 in Magway Region > 2.27 % and 383 in Rakhine State >0.57 % and 96 in Shan State > 0.37 % and 62 in Mon State from *C. longa* respectively.

One organic compound, curcumin (orange needles, 0.6248 %, R_f-0.44, mpt. 175 °C) was major chemical constituent from crude extract of the rhizome by using silica gel column chromatographic separation technique.

According to my research findings, curcumin content and color value were found to be highest in Kayin state and that of Mon State sample are the lowest. With these results, Na-nwin from Kayin State can be used as medicinal purpose, especially cancer diseases.

Acknowledgments

The authors would like to express their profound gratitude to the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research and Myanmar Academy of Arts and Science for allowing to present this paper.

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