Identification of Isolated Curcumin from Rhizomes of *Curcuma longa* L. (Na-nwin) and Investigation of Antimicrobial Activity of the Various Crude Extracts

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Abstract

The medicinal plant turmeric, *Curcuma longa* L. (Na-nwin) is known as some medicinal uses and biological activities in Myanmar. The research focused on isolation of some organic constituents, identification of isolated compound and antimicrobial activity of the various crude extracts of Na-nwin Rhizomes. Preliminary phytochemical tests indicated that the presence of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, terpenoids, reducing sugars, steroids and tannins whereasalkaloids were not detected. The elemental analysis of dried powdered sample was also carried out by EDXRF technique. The high contents of Fe (86.0 %) and K (9.35 %) were observed. Isolated compound was obtained from 95 % EtOH extract of Na-nwin rhizomes and it was identified as curcumin by melting point determination, thin layer chromatography and modern spectroscopic methods such as UV and FT IR. In addition, investigation of antimicrobial activity on various crude extracts (PE, EtOAc and EtOH) of Na-nwin rhizomes was done by agar well diffusion method against six microorganisms: *Agrobacterium tumefaciens, Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae, Staphylococcus aureus* and *Candida albicans*. From these results, it was found that ethanol extract of Na-nwin rhizomes significantly exhibited antimicrobial activity when compared with the other extracts. So, the rhizomes of Na-nwin may be used for the treatment of diseases caused by bacteria.

Keywords: *Curcuma longa* L., phytochemical constituents, elemental analysis, antimicrobial activity, agar well diffusion method

Introduction

The origin of the plant *Curcuma longa* L., which belongs to Zingiberaceae family is from India. The plant is distributed throughout tropical and subtropical regions of the world, being widely cultivated in southeast Asian countries. Turmeric, i.e., the ground rhizomes of *Curcuma longa* L., has a long history of use in food as a spice, mainly as an ingredient in many varieties of curry powders and sauces, where curcumin from turmeric is a main coloring substance. In Indian systems of medicine, turmeric is used to some extent as a stomachic, tonic and blood purifier. It has also been employed to stimulate biliary secretion and to treat gallstone (Tyler *et al*., 1981).

Major constituents of rhizomes are pale yellow to orange-yellow volatile oil (6 %) composed of a number of monoterpenes and sesquiterpenes, including zingiberene, curcumene, α- and β-turmerone among others. The coloring principles (5 %) are curcuminoids, 50-60 % of which are a mixture of curcumin, monodemethoxycurcumin and bisdemethoxycurcumin (WHO, 1999).

Curcumin, a major yellow pigment of turmeric obtained from powdered rhizomes of the plant *Curcuma longa* is commonly used as coloring agent in foods, drugs and cosmetics. The active ingredient in turmeric is curcumin, which is approximately 2 % by weight of the root of turmeric. The long list of uses include antiseptic, analgesic, anti-inflammatory, antioxidant, antimalarial, insect-repellant, and other activities associated to turmeric. The research work is concerned with the determination of phytochemical constituents, minerals, isolation of some organic constituents and identification of isolated compound by melting point

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determination, UV-visible, FT IR spectroscopic techniques and investigation of antimicrobial activity of Na-nwin rhizomes by agar well diffusion method. The photographs of plants, flowers and rhizomes of Na-nwin are shown in Figure 1.

(a) (b) (c)

Figure 1 Photographs of Na-nwin (a) plants (b) flowers and (c) rhizomes

Materials and Methods

Collection and Preparation of the Samples

In this study, selected rhizomes of *Curcuma longa* (Na-nwin) were collected from Hinthata Township, Ayeyarwaddy Region, Myanmar, during the months of November and December, in the year of 2010. The plant sample was identified as *Curcuma longa* L. by authorized botanist at Botany Department, University of Yangon, Myanmar.

The collected fresh rhizomes were cleaned by washing with water and air dried at room temperature for two weeks. The dried rhizomes were cut into a small pieces and were made into powder by using grinding machine. The dried powdered sample was separately stored in the air tight containers to prevent moisture and other contaminations.

Preliminary Phytochemical Tests

The air-dried powdered samples were subjected to preliminary phytochemical test in order to find out the types of phytoorganic constituents such as alkaloids (Trease and Evans, 1980), α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds (Marini *et al*., 1981), reducing sugars, saponins, terpenoids, steroids and tannins (Shriner *et al*., 1980).

Preparation of Various Crude Extracts

The dried powdered samples (15 g) were extracted with (150 cm^3) of pet-ether, ethyl acetate and ethanol in separate conical flask, respectively for at least 7 days and then filtered. The filtrates were evaporated by using rotatory evaporator and desiccated. Then the dried extracts were weighed. Each extract was stored in refrigerator for screening of antimicrobial activity.

Determination of Mineral Contents in Na-nwin Rhizomes

Mineral contents from the rhizomes of Na-nwin were measured at the Department of Physics, University of Taunggyi, Myanmar by applying Energy Dispersive X-ray Fluorescence (EDXRF) technique.

Isolation of Curcuminoids from Crude Curcuminoid mixture

The dried powdered sample of Na-nwin rhizomes (100 g) was extracted with 95 % ethanol for one week at room temperature. One hundred gram of turmeric powder yielded about fifteen grams of laboratory made crude curcuminoids.

The crude curcuminoids were then purified by washing with petroleum ether. The obtained precipitate (5 g) is the crude curcuminoid mixture.

The obtained crude curcuminoid mixture (0.3) g was chromatographed on silica gel (3.5) g by elution with $CHCl_3$ only solvent system. A total of 40 fractions were collected from three different eluents of increasing polarity, i.e, $CHCl_3$ only, $CHCl_3$: MeOH, 98:2 and 97:3, v/v. Finally, three main fractions FII (F_{14}-F_{22}), FIII (F_{25}-F_{30}), FIV (F_{33}- F_{40}) were collected.

Characterization and Identification of Isolated Compound

The isolated compound was characterized by determination of some physical properties such as melting point, R_f values and solubilities in some solvents and by some colour tests. The structure of isolated compound was elucidated and identified by UV-visible and FT IR spectroscopies which were recorded at Universities' Research Center, University of Yangon, Myanmar.

Screening of Antimicrobial Activity of Various Crude Extracts of Na-nwin Rhizomes by Agar Well Diffusion Method

The antimicrobial activity of various crude extracts (PE, EtOAc and EtOH) was determined against six strains of microorganisms such as *Saccharomyces cerevisiae, Agrobacterium tumefaciens, Staphylococcus aureus, Pseudomonas fluorescens, Candida albicans* and *Escherichia coli* by employing agar well diffusion method at Botany Department, Kyaukse University, Mandalay Region, Myanmar.

A loop full of bacterial strain was inoculated in nutrient broth in a conical flask and incubated for 24 hours to get active strain by using agar well diffusion method. Agar was poured into petri dishes. After solidification of tests strains were inoculated in the media separately. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer. The extract or compound was introduced into well and plates were incubated at 37°C for 24 hours. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition. A control with standard antibiotic was kept for all test strains and the control activity was deducted from the tests and results were recorded (Perez, 1990).

Results and Discussion

Types of Phytochemicals Present in Sample

In order to find out the types of phytochemical constituents that present in the rhizomes of Na-nwinphytochemical tests were preliminarily carried out according to the appropriate reported methods.

According to these results, it was observed that α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, terpenoids, steroids and tannins were found to be present in the rhizomes of Na-nwin. However, alkaloids were found to be absent in rhizomes samples.

Determination of Mineral Contents in Na-nwin Rhizomes

The rhizomes of Na-nwin were examined for elemental contents (qualitatively) by EDXRF method. It was found that macronutrients Fe, K, Pb and Dy are present in the rhizomes of Na-nwin. Other trace elements (micronutrients):

Ca, P, Mg, Al, Cl and Ta are also present in Na-nwin rhizomes. The high contents of Fe (86.0 %) and K (9.35 %) were observed. These observed results were shown in Table 1 and Figure 2.

No.	Elements	Relative Composition (%)
1	Iron (Fe)	86.00
2	Potassium (K)	9.35
3	Lead (Pb)	1.10
4	Dysprosium (Dy)	1.05
5	Calcium (Ca)	0.743
6	Phosphorous (P)	0.499
7	Magnesium (Mg)	0.483
8	Aluminium (Al)	0.260
9	Chlorine (Cl)	0.243
10	Tantalum (Ta)	0.146

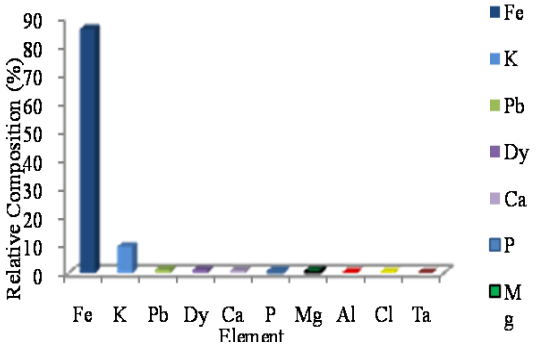


Figure 2 Histogram showing the relative percentages of minerals contained in Na-nwin rhizomes

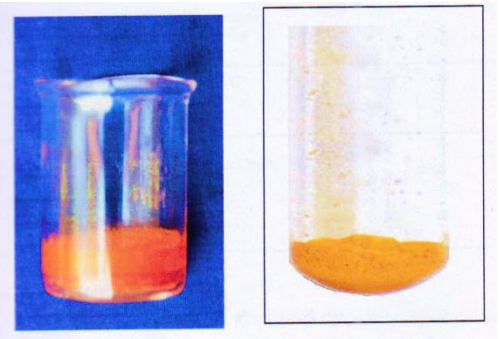
Isolation of Pure Curcumin from Crude Curcumin in Na-nwin Rhizomes

The obtained crude curcuminoid mixture (0.3 g) were separated column-chromatographically on silica gel GF_{254} adsorbent by increasing the polarity of eluent, $CHCl_3$ only, $CHCl_3$ and MeOH (98:2 to 97:3, v/v). The collection of fraction was performed by visualizing in day light and their TLC behavior.

The three crude curcuminoids (compound I, compound II and compound III) were isolated. Compound I, crude curcumin (orange yellow crystals powder, 0.1 % yield, R_f = 0.59; $CHCl_3$ only, m.pt = 183 ºC), compound II, crude demethoxycurcumin (yellow crystals powder, 0.02 % yield, R_f = 0.38, $CHCl_3$: MeOH ≡ 98:2 v/v) and compound III crude bisdemethoxycurcumin (yellow crystals powder, 0.005 % yield), R_f = 0.27, $CHCl_3$: MeOH ≡ 97:3 v/v) were isolated.

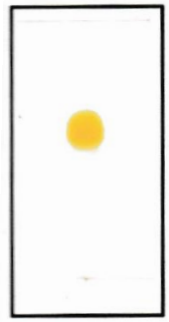
The obtained crude curcumin (100 mg) was dissolved in $CHCl_3$ solvent (2 mL). The $CHCl_3$ soluble crude curcumin was added 2N NaOH solution (4 mL) and the mixture solution was shaken. The solution was formed into two layers. After separating the two layers of solution, the solution in the upper layer was added 2N HCl until giving the precipitate.

The residue was obtained by filtration, it was washed with H_2O and was dried. After drying, the pure curcumin was obtained as yellow crystals powder (50 mg, 0.05%). Pure curcumin obtained from 95 % EtOH crude extract was used to identify the structure by melting point, TLC and spectrometric methods. The photographs of crude curcumin and pure curcumin are shown in Figure 3. Thin layer chromatogram of pure curcumin is shown in figure 4.



(a) (b)

Figure 3 Photographs of (a) crude curcumin (b) pure curcumin



Solvent system : $CHCl_3$ only
Visualization : day light (yellow colour)
R_f value : 0.59

Figure 4 Thin layer chromatogram of pure curcumin

Out of three isolated compounds, only one compound could be structurally identified. The melting point of the isolated compound was determined by applying Gallenkamp melting point apparatus. Curcumin isolated as yellow crystals powder from 95 % EtOH crude extract has the melting point of 183 ºC, which consistent with reported value of curcumin (183ºC). It was soluble in pet-ether, ethanol and methanol, however insoluble in water. According to iodine vapor test, brown colour was observed, indicating that phenolic OH group was present in curcumin. The R_f value of curcumin was 0.59 in $CHCl_3$ only solvent system.

The structure of curcumin was identified by using modern spectroscopic methods such as UV-visible and FT IR spectroscopy. UV-visible spectra of pure curcumin was illustrated in Figure 5. The wavelength of maximum absorption was observed at 428 nm, which consistent with λ_{max} value (422 nm) of curcumin reported in literature (Kosuge, *et al*., 1985). There were two absorption bands appeared at 264 nm and 428 nm with high intensities in EtOH were respectively due to the $\pi - \pi^*$ transition and n - π^* transition. In addition, the absorption maxima (464 nm) occurred to be shifted to longer wavelength in the presence of NaOH. This observation revealed that there was phenolic or enolic group present in curcumin.

The functional group present in the isolated compound could be assigned from its FT IR spectrum. Absorption band at 3356 cm^{-1} was due to –OH stretching vibration of phenolic hydroxyl groups. Aliphatic C-H stretching vibrations of CH_3 and CH_2 groups were found at 2931 cm^{-1} and 2860 cm^{-1}, respectively, conjugated C=O group and carbonyl stretching vibration of keto-enol system was observed at 1689 cm^{-1} and 1624 cm^{-1} and C=C stretching vibrations of aromatic ring system were at 1589 cm^{-1}, 1512 cm^{-1} and 1458 cm^{-1}, respectively. Absorption band at 1373 cm^{-1} was due to bending vibration of aliphatic chain. Asymmetric and symmetric stretching vibrations of C-O-C group were found at 1286 cm^{-1} and 1033 cm^{-1}, respectively. Out of plane bending vibration of trans olefinic C-H was assigned at 970 cm^{-1}, and that of aromatic C-H were at 870 cm^{-1} and 813 cm^{-1}, respectively. Figure 6 represents the FT IR spectrum of curcumin. From the results of melting point determination, TLC examination and spectroscopic evidences the isolated compound was defined as curcumin.

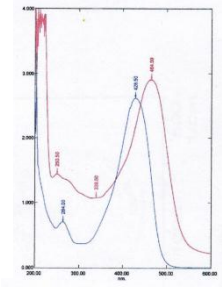


Figure 5 UV-visible spectra of curcumin

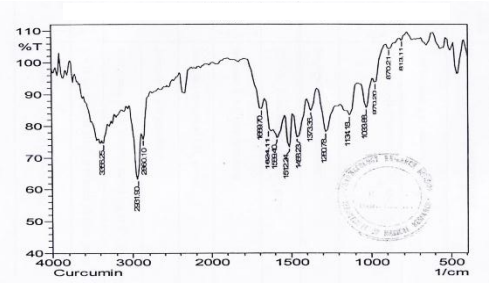


Figure 6 FT IR spectrum of curcumin

Antimicrobial Activity of the Various Crude Extracts of Na-nwin Rhizomes

Screening of antimicrobial activity of various crude extracts such as pet-ether, ethyl acetate and ethanol extracts from Na-nwin rhizomes was determined by agar well diffusion method. In this investigation, the samples were tested on six microorganisms such as *Agrobacterium tumefaciens, Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae, Staphylococcus aureus* and *Candida albicans* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameters indicate the higher the antimicrobial activity. The inhibition zone diameters resulted from antimicrobial activity of various crude extracts of Na-nwin rhizomes are summarized in Table 2 and Figure 7 and the observed inhibition zones are shown in Figure 8.

According to the results, it was found that ethanol extract of Na-nwin rhizomes exhibited the most pronounced antimicrobial activity against five organisms: *Agrobacterium tumefaciens, Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae and Staphylococcus aureus* with the inhibition zone diameter ranged between 25 mm -37 mm. In addition, pet-ether extract (inhibition zone diameter 15 mm -31 mm), ethyl acetate extract (inhibition zone diameter 18 mm -30 mm) exhibited antimicrobial activity against above five species of microorganisms tested. But antimicrobial activities of pet-ether, ethyl acetate and ethanol crude extracts of Na-nwin rhizomes did not show against *Candida albicans.* It can be observed that ethanol extract of Na-nwin rhizomes significantly exhibited antimicrobial activity when compared with the other extracts.

Table 2. Antimicrobial Activity of Various Crude Extracts of Na-nwin Rhizomes

No	Test Organisms	Inhibition Zone Diameter (mm)		
		PE	EtOAc	EtOH
1	*Agrobacterium tumefaciens*	15.09	18.01	28.99
2	*Escherichia coli*	17.28	19.23	37.69
3	*Pseudomonas fluorescens*	31.18	30.46	27.74
4	*Saccharomyces cerevisiae*	27.11	18.94	25.43
5	*Staphylococcus aureus*	22.71	18.49	30.02
6	*Candida albicans*	-	-	-

Agar well diameter = 8 mm

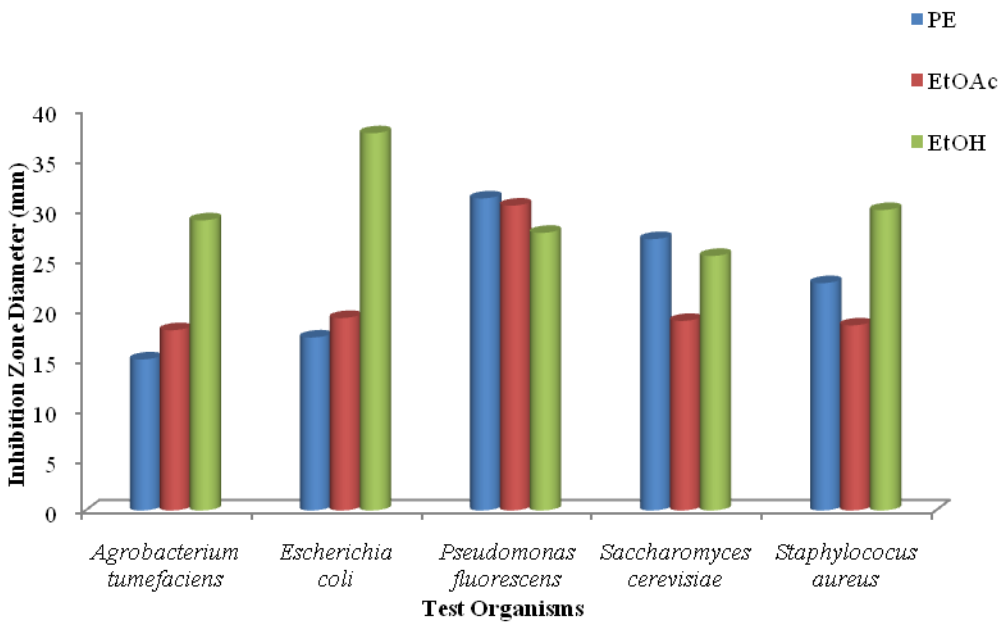


Figure 7 Histogram of inhibition zone diameters (mm) of various crude extracts of Na-nwin rhizomes on six microorganisms

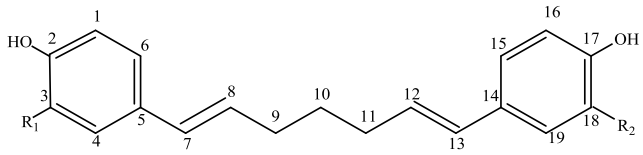
PE extract *Saccharomyces cerevisiae* | EtOAc extract *Pseudomonas fluorescens* | EtOH extract *Escherichia coli*

Figure 8 Photographs of inhibition zones of various solvent extracts of Na-nwin rhizomes

Conclusion

Preliminary phytochemical tests revealed the presence of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, terpenoids, steroids and tannins in the rhizomes of *Curcuma longa* L. Qualitative elemental analysis of dried powdered rhizomes sample was done by EDXRF spectrometer. It was found that macronutrients such as Fe, K, Pb, Dy,Ca,P, Mg and Al as trace elements were present in the rhizomes of Na-nwin. Among these elements, the contents of iron (86.0 %), potassium (9.35 %) in the sample as higher constituents. Curcumin (50 mg, 0.05 %), crude demethoxycurcumin (20 mg, 0.02 %) and crude bisdemethoxycurcumin (5 mg, 0.005 %) from the rhizomes of Na-nwin were isolated by using solvent extraction and column chromatographic method. Isolated compound; curcumin (50 mg, 0.05 %) was obtained in the pure form as yellow crystal powders. The isolated curcumin was identified by melting

point determination, thin layer chromatography and modern spectroscopic methods such as UV, FT IR. In addition, antimicrobial activity of crude extracts (PE, EtOAc and EtOH) of Na-nwin rhizomes was screened by using agar well diffusion method against six microorganisms. The ethanol extracts were found to exhibit the most pronounced antimicrobial activity against *Agrobacterium tumefaciens, Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae, and Staphylococcus aureus.* It was observed that ethyl acetate extract exhibited activity on *Pseudomonas fluorescens.* Pet-ether extract exhibited activity on *Pseudomonas fluorescens, Saccharomyces cerevisiae, and Staphylococcus aureus* .But all crude extracts gave no activities on *Candida albicans.* Therefore, ethanol extract of Na-nwin rhizomes can be considered to be biologically active.



Curcumin

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