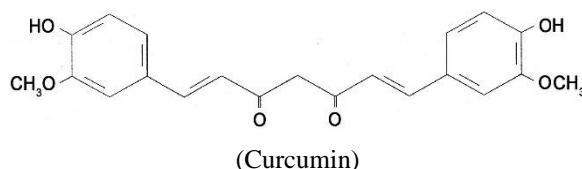


INVESTIGATION OF SOME BIOACTIVITIES AND IDENTIFICATION OF CURCUMIN FROM THE RHIZOME OF *Curcuma longa* L. (NA-NWIN)

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ABSTRACT

In the present research, the Myanmar indigenous medicinal plant, the rhizome of *Curcuma longa* L. (Na-nwin in Myanmar) was chosen for investigation of some bioactivities and identification of isolated compound by spectroscopic methods. Firstly, the phytochemical constituents and elemental contents were carried out. The antimicrobial activity of ethyl acetate, ethanol and watery extracts was determined by Agar Well Diffusion method against six species of microorganisms namely *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *E. coli* and *Candida albicans*. It was found that the ethyl acetate extract showed the highest antimicrobial activity than the ethanol and watery extracts. In addition, the antitumor activity of crude extracts was investigated by Potato Crown Gall test. The three crude extracts can exhibit the growth of tumor. Among the three crude extracts, the most active ethyl acetate extract was selected for isolation of compound by column chromatographic separation technique. The isolated compound, curcumin (0.09 %) was identified by spectroscopic methods.



Keywords: *Curcuma longa* L., antioxidant activity, antimicrobial activity, antitumor activity, curcumin

INTRODUCTION

Curcuma longa L., botanically related to ginger (Zingiberaceae family), is a perennial plant having a short stem with large oblong leaves and bears ovate, pyriform or oblong rhizomes, which are often branched or brownish-yellow in colour. It is extensively used as a spice, food preservative and coloring material in India, China and South East Asia. It has been used in traditional medicine as a household remedy for various diseases including anti-inflammatory, antioxidant, anticarcinogenic, antifertility, antidiabetic, antibacterial, antifungal, antiviral, hypotensive and hypocholesteremic activities. Curcumin, the main yellow bioactive component of turmeric has been shown to have a wide spectrum of biological actions and isolated from the rhizome of the plant *C. longa*. In Myanmar, *C. longa* is widely cultivated from sea level to 1200 m. The photograph of rhizome of *C. longa* L. is shown in Figure 1. The present research has focused on the investigation of some bioactivities and identification of curcumin from the rhizome of *Curcuma longa* L. (Na-nwin).

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Figure 1. Rhizome of *Curcuma longa* L. (Na-nwin)

MATERIALS AND METHODS

In this research work, the rhizome of *Curcuma longa* L. (Na-nwin) was collected from Thandaung Myo Thit, Kayin State during December, 2017 and identified at Botany Department, Taungoo University. The collected sample was washed with water and dried at room temperature for one week. The dried sample was cut into small pieces and ground into powder. The powdered sample was stored in airtight container to prevent moisture changes and other contaminations.

All chemicals used in this research were analar grade (BDH) and the instruments consisted of a Shimadzu EDX-700 Spectrometer, Shimadzu UV-240 Spectrophotometer, Shimadzu FT IR- 8400 Spectrophotometer, Bruker 300 MHz and 75 MHz Spectrometers.

Qualitative Elemental Analysis of Rhizome of *Curcuma longa* L. by EDXRF Spectrometry

For this measurement, pellet of the sample (2.5cm diameter) was first made. X-ray fluorescence spectrometer (Shimadzu EDX-700) can analyze the element from Na to U under vacuum condition. The sample was placed in the same chamber and pumped up to vacuum. The vacuum pressure was about 38 Pa and the detector temperature was about 170 °C. Therefore, liquid nitrogen needs to be added at the time of analysis. The measurement condition of X-ray spectrometer was used Rh target. The sample was run for a counting time of about 100s and the spectrum obtained was stored and analyzed in PC based multi-channel analyzer using EDX-700 software.

Investigation of Bioactivities of Rhizome *Curcuma longa* L.

Investigation of Antimicrobial Activity by Agar Well Diffusion Method

Agar well diffusion method was employed for determining the antimicrobial activity of the ethyl acetate, ethanol and watery extracts of rhizome of *Curcuma longa* L. Two small holes of 10 mm diameter each were cut out in the inoculated agar to place the tested samples. The volume of each sample placed in each hole was 0.1 mL. The petri dish was then incubated at 27°C for 24 h and the diameter of clear inhibition zone appeared around the hole were measured.

Investigation of Antitumor Activity by Potato Crown Gall Test

Tubers of moderated size obtained from fresh potato were surface sterilized by immersion in 50% sodium hypochlorite (clorox) for 20 min. The ends were removed and soaked for 10 min more in clorox. A core of the tissue was extracted from each tuber by using surface sterilized (ethanol and flame) 1.0 cm wide cork borer. Pieces of 2 cm were removed

from each end and discarded. The remainder of the cylinder is cut into 0.5 cm thick discs with a surface sterilized cutter. The discs were then transferred to agar plates (1.5g of agar was dissolved in 100 mL distilled water, autoclaved for 20 min at 121°C, 20 mL poured into each petri dish). Each plate contained three potato discs and 3 plates were used for each simple dilution.

Sample (0.15g, 0.1g, 0.05g) of crude extracts were dissolved in dimethyl sulphoxide (DMSO) (2mL) and filtered through Millipore filter (0.22 µm) into sterile tube. This solution (0.5 mL) was added to sterile distilled water (1.5 mL) and broth culture (2 mL) of *A.tumefaciens* strains. Controls were made in this way, DMSO (0.5mL) and sterile distilled water (1.5mL) were added to the tube containing broth culture (2 mL) of *A.tumefaciens* strains. By using a sterile disposable pipette, one drop (0.5 mL) from these tubes was used to inoculate each potato discs spreading it over the discs surface. After inoculation, petri dishes were sealed by paraffin and incubated at 27-30°C for 3 days. Tumors were observed on potato discs after 3 days under stereomicroscope followed by staining with Lugol's solution (5% I₂ and 10% KI) after 30 min and compared with control. The antitumor activity was examined by observation of crown gall produced or not.

Isolation and Identification of Phytoconstituent from the Ethyl Acetate Crude Extract

The ethyl acetate crude extract was subjected to chromatography over a silica gel column. The column was initially eluted with chloroform:methanol(9:1 v/v) solvent system and the fractions were collected at the rate of one drop per second. The polarity of eluting solvent was gradually increased by addition of decreasing amount of chloroform in methanol. A quantity of 20 mL was collected for each fraction and chromatographic separation was monitored by TLC. Fractions that showed similar TLC pattern were combined to provide four main fractions. Fraction F₂ provided compound 1 as orange-yellow crystals. The isolated compound was characterized by its spectral data, Shimadzu UV-240 Spectrophotometer, Shimadzu FTIR-8400 Spectrophotometer, Bruker 300 MHz and 75 MHz Spectrometers.

RESULTS AND DISCUSSION

Qualitative Elemental Analysis of Rhizome of *Curcuma longa* L. by EDXRF Spectrometry

In this work, the mineral elements present in rhizome of *C. longa* were determined by EDXRF spectrometer. It can be found that the content of K (80.83 %) is more significant than the other elements such as Ca, Fe, Si, Mn, S, P, Rb, Zn and Cu. The significant level of potassium found in sample ensures a proper balance between the fluids in the body and also counteracts the effects of blood stream. Potassium is a vasodilator, meaning that it relaxes blood vessels and by rigidity, it increases blood flow and reduces the strain on the cardiovascular system.

Antimicrobial Activity of Crude Extracts of Rhizome of *Curcuma longa* L.

The antimicrobial activity of ethyl acetate, ethanol and watery extracts of rhizome of *Curcuma longa* L. was screened by Agar Well Diffusion method on *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aeruginosa*, *E.coli* and *Candida albicans*. According to the results, it was found that these three extracts exhibited antimicrobial activity against all test species. In addition, ethyl acetate extract showed the strong antimicrobial activity with inhibition zone diameter 23-30 mm than that of other crude extracts. Therefore, the rhizome of *C. longa* may be effectively used for the treatment of diseases infected by the microorganisms such as skin infection, wounds, diarrhoea and dysentery.

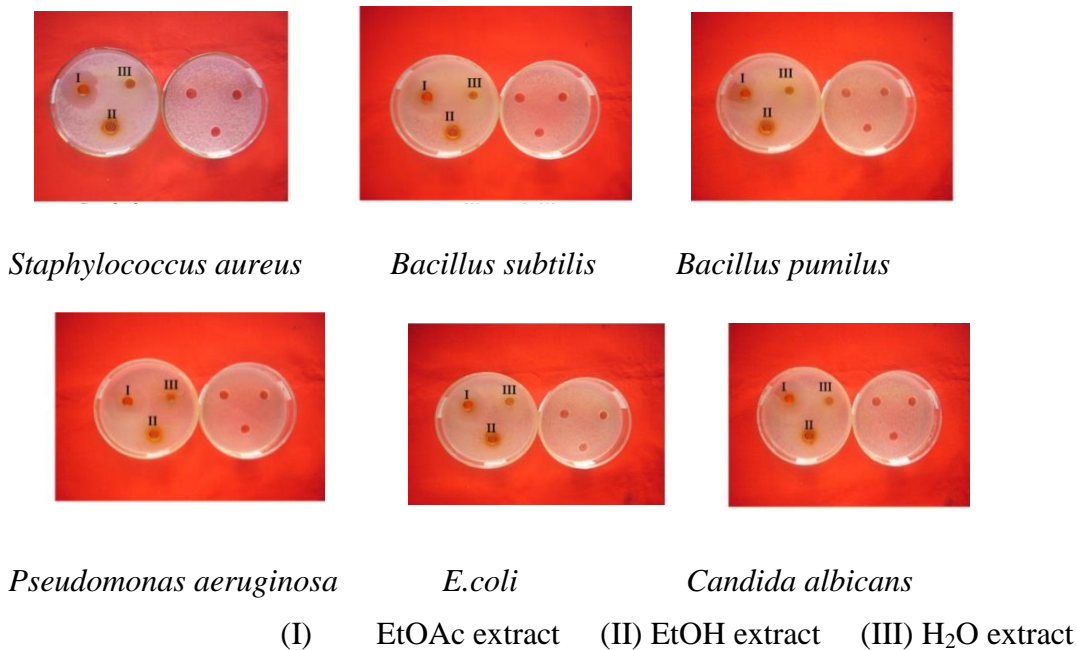


Figure 2. Antimicrobial activity of crude extracts of rhizome of *Curcuma longa* L.

Antitumor Activity of Crude Extracts of Rhizome of *Curcuma longa* L.

The antitumor activity of ethyl acetate, ethanol and watery extracts of rhizome of *C. longa* L. was investigated by Potato Crown Gall test with *A. tumefaciens* isolated from *Sandoricum koetjape* Merr. (Thitto). For inoculation of the potato discs, 48 h broth cultures containing 5×10^9 cells/mL were used. The tested samples were dissolved in DMSO and the diluted samples were mixed with the bacterial culture for inoculation. After preparing the inoculations, the bacterial suspensions were inoculated on the cleaned and sterilized potato discs, and incubated for 3 days at room temperature. After that, the tumors on potato discs were checked by staining the Knob with Lugol's solution. In control disc, formation of white Knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. If the test sample has antitumor activity, no tumor on the potato disc was observed and its surface remained blue as shown in Figures 3, 4 and 5. In this study, it was found that ethyl acetate, ethanol and watery extracts of rhizome of *C. longa* were effective in preventing the tumor formation with the doses of 0.15, 0.1 and 0.05 g/disc in *in vitro* potato disc assays.

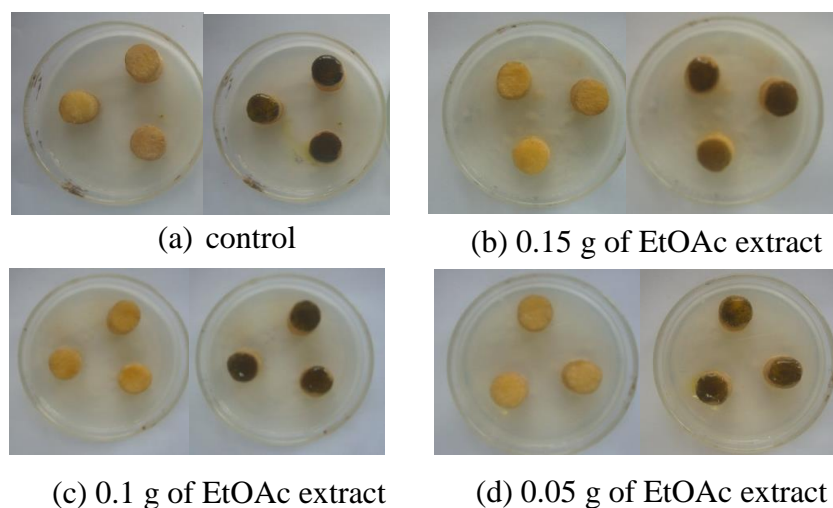
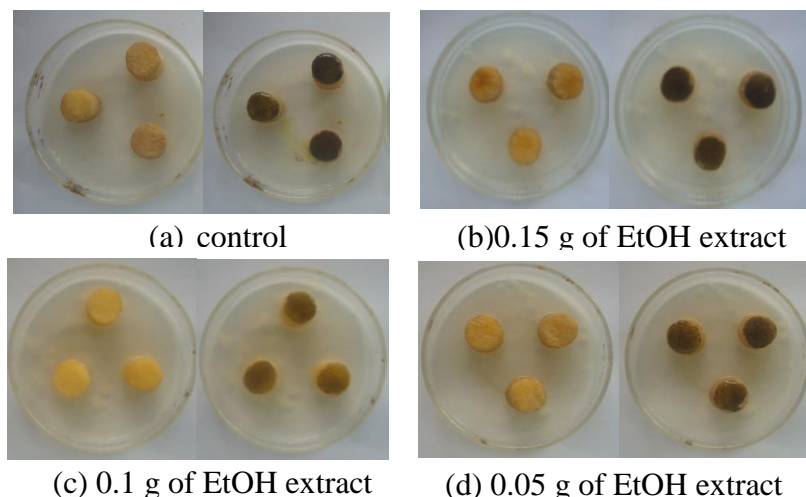
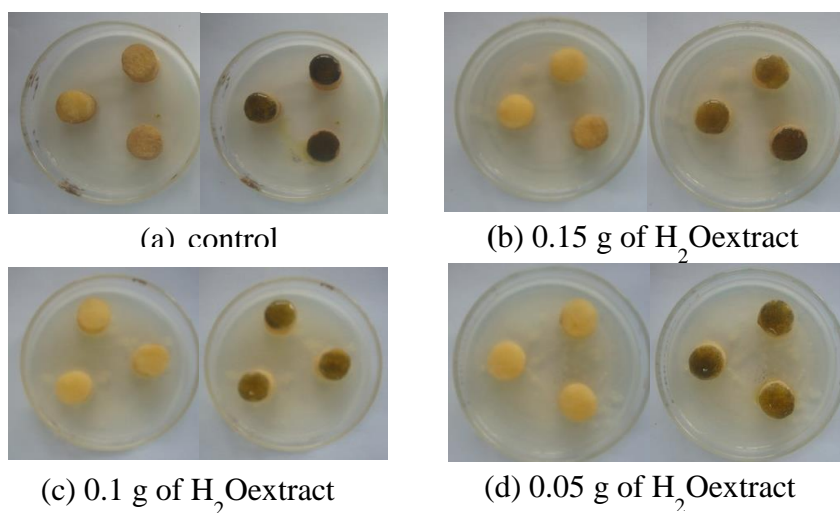


Figure3. Tumor inhibition by EtOAc extract of rhizome of *Curcuma longa*L.Figure4. Tumor inhibition by EtOH extract of rhizome of *Curcuma longa*L.Figure5. Tumor inhibition by H₂O extract of rhizome of *Curcuma longa*L

Identification of Isolated Compound

The melting point of isolated compound was found to be 183°C and which is consistent with reported value of curcumin. Ultraviolet spectrum of isolated compound measured in methanol solvent was illustrated in Figure 6. The wavelength of maximum absorption was observed at 425 nm, which consists with λ_{\max} value of curcumin reported in literature.

The functional groups present in the isolated compound could be assigned from its FTIR spectrum described in Figure 7. Absorption band at 3374 cm^{-1} was due to $-\text{OH}$ stretching vibration of phenolic-OH group. Aliphatic C-H stretching vibrations of CH_3 and CH_2 groups were found at 2944 cm^{-1} and 2843 cm^{-1} , respectively. Carbonyl stretching vibration of keto-enol system was observed at 1629 cm^{-1} and C=C stretching vibrations of aromatic ring system were at 1603 cm^{-1} , 1510 cm^{-1} and 1428 cm^{-1} , respectively. Absorption band at 1375 cm^{-1} was due to bending vibration of aliphatic chain. Asymmetric and symmetric stretching vibration of

C-O-C groups were found at 1282 cm^{-1} and 1023 cm^{-1} , respectively. Out of plane bending vibration of trans olefinic C-H was assigned at 961 cm^{-1} , and that of aromatic C-H were at 857 cm^{-1} , 813 cm^{-1} and 715 cm^{-1} , respectively.

^1H NMR spectrum of isolated compound was taken in CDCl_3 and CD_3OD solvent mixture with 300 MHz spectrometer, which was shown in Figure 8. The total 20 protons and their environments could be determined from this spectrum. The singlet sharp signal at δ 3.92 indicated for the 6 protons of two methoxygroup and the broad singlet at δ 4.75, for the two protons of two hydroxyl groups. The methylene proton of keto-enol system was observed at δ 5.83 as singlet. The doublet at δ 6.54 was for the two olefinic protons and the doublet at δ 7.58 for the two conjugated olefinic protons. The doublet at δ 6.86 exhibited for two protons ortho to the $-\text{OH}$ group of aromatic ring. The doublet of doublet at δ 7.07 showed for the two protons meta to the $-\text{OH}$ group of aromatic ring and the doublet at δ 7.15 indicated for the two protons meta to the $-\text{OH}$ group of aromatic ring.

^{13}C NMR spectrum of isolated compound showed in Figure9 indicated the total 21 carbon atoms. The methylene carbon of keto-enol system was found at δ 49 and methoxy carbon of $-\text{OCH}_3$ group at δ 56. Aromatic C-H carbons which were meta and ortho to $-\text{OH}$ group were observed at δ 111, 124 and 116, respectively. δ 122 and 142 were assigned as the olefinic C-H carbons which were near to the carbonyl and conjugated with the carbonyl group. The aromatic carbons were found at δ 128, 148 and 150, respectively. Finally the carbonyl carbons were assigned at δ 184. From the results of melting point determination and spectroscopic evidences, the isolated compound was defined as curcumin.

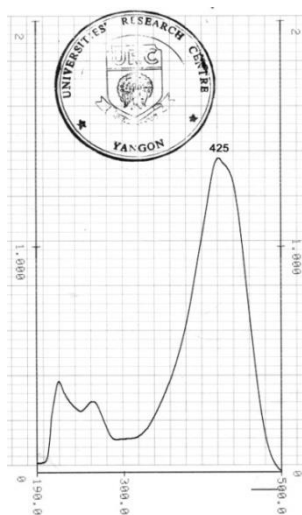


Figure 6. UV spectrum of isolated compound

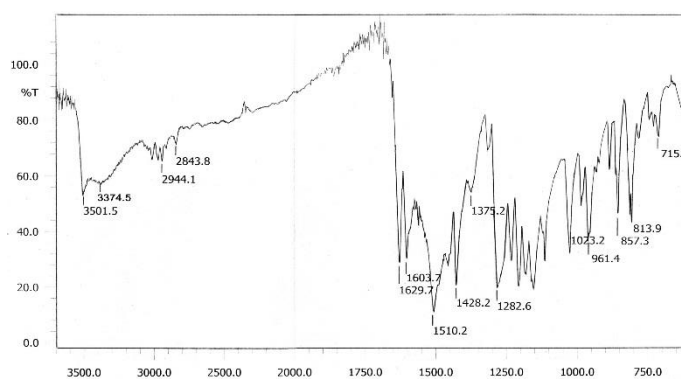


Figure 7. FT IR spectrum of isolated compound

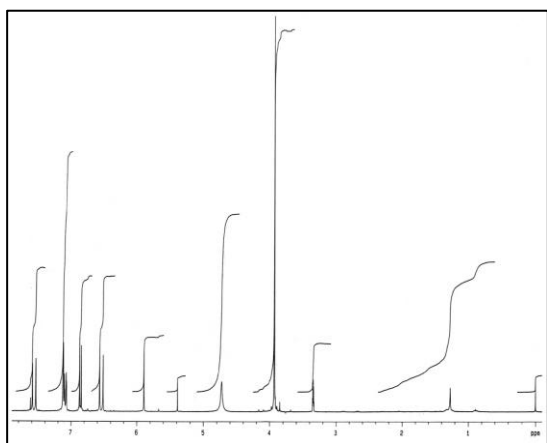


Figure 8. ^1H NMR spectrum of isolated compound

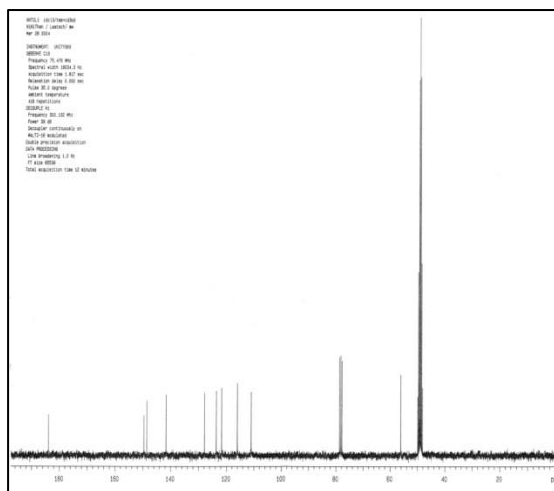


Figure9. ^{13}C NMR spectrum of isolated compound

CONCLUSION

In the qualitative elemental analysis of rhizome of *Curcuma longa* L. by EDXRF, K is more significant than other elements such as Ca, Fe, Si, Mn, S, P, Rb, Zn and Cu. Screening of antimicrobial activity of ethyl acetate, ethanol and watery extracts of rhizome of *C.longa* by Agar Well Diffusion method, ethyl acetate extract showed the strong antimicrobial activity with inhibition zone diameter range between 23-30 mm. Antitumor activity of crude extracts of *C.longa* was also screened by Potato Crown Gall test. In this study, these three crude extracts were effective in preventing the tumor growth with the doses of 0.15 g, 0.1 g and 0.05 g/disc. According to these observations, it can be inferred that rhizome of *C.longa* possesses antimicrobial and antitumor activities. Therefore, the rhizome of *C.longa* may be effectively used for the treatment of diseases infected by the microorganisms such as skin infection, wounds, diarrhea, dysentery and several forms of cancer in Myanmar traditional medicine.

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