Investigation on Antimalarial Activity of Rhizome of *Zingiber cassumunar* Roxb. (Meik-tha-lin-oot)

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This research aims to investigate the antimalarial activity of rhizome of *Zingiber cassumunar* Roxb. (Meik-tha-lin-oot). The preliminary phytochemical investigation revealed the presence of α-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch and terpenoids. The various crude extracts such as PE (7.11%), 95% EtOH (3.44%), EtOAc (1.64%) and H_2O (1.44%) extracts were prepared by successive solvent extraction method. Curcumin (orange amorphous crystal, 0.00317% yield, M.pt=183°C) was isolated from EtOAc crude extract by using thin layer and column chromatographic separation techniques and identified by comparing with UV and FTIR spectral data of the standard curcumin. *In vitro* antimalarial activities of curcumin, ethanol and watery extracts were screened by using the reported method. The ethanol and watery extracts had no antimalarial activity (EC_{50} values=398.07 μg/ml and 501.00 μg/ml) whereas the isolated compound, curcumin was found to inhibit the growth of *Plasmodium falciparum* (EC_{50}=101.74 μg/ml). On the other hand, the isolated compound, curcumin exhibited the moderate antimalarial activity and may be used as a potential compound to be tested for newer antimalarial agent.

Keywords: Curcumin, *Zingiber cassumunar* Roxb., UV, FTIR, Antimalarial activity, EC_{50} values

INTRODUCTION

Malaria is one of the most common major health problems in tropical and developing countries of sub-Saharan Africa and South East Asia including India. It is a major killing disease which is responsible for the death of millions of children, pregnant women and adults.[1] The aim of this study was to explore the potential newer, effective, and safe herbal compound and hoped to complement the existing therapy by the finding from this study. *Zingiber cassumunar* Roxb. (Figure 1) is a member of Zingeraberaceae family. It is an erect herb with subterranean stem called rhizome. The rhizome is jointed but much larger than *Zingiber officinale*, when fresh it is of deep yellow colour possessing a strong camphoraceous smell, warm, spicy and bitterish taste. The pseudo stem is made up of leave sheaths. The leaflets are bifarious, approximate, sessile on their sheaths, linear-linceolate, deep green above; villous and paler underneath.[2] Plant native is Thailand, Indonesia, India and Myanmar.[3]

Z. cassumunar had been reported to possess many pharmacological activities such as the anti-inflammatory[4], analgesic, antipyretic[3], antiviral, antiseptic, antibacterial and anti-oxidant activities.[5]

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Flower Rhizomes

Fig. 1. Photographs of flower and rhizomes of *Zingiber cassumunar* Roxb. (Meik-tha-lin-oot)

Z. cassumunar has traditionally been widely used to beautify the skin, to ward off asthma, chronic colds, nausea poultice, decoction, and medicinal massage treatment. It is used in relieving abdomen pain, headache, stomachache, anodyne, constipation, colic, cramps, constipation, fever, flatulence, gonorrhoea, jaundice, malaria, numbness, parturition and vermifuge. Moreover, it has also been used in joint and muscle inflammation and helps to reduce fever generation. It is also used as topical treatment for sprains, contusions, joint inflammations, muscular pain, abscesses, and similar inflammation-related disorders.[6, 7]

MATERIALS AND METHODS

Sample collection

The rhizomes of *Zingiber cassumunar* Roxb. (Meik-tha-lin-oot, MTLO) were collected from Chaungzone Township, Mon State. After washing with water, the collected samples were dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. These powdered samples were stored in air-tight containers.

Preliminary phytochemical analysis

The preliminary phytochemical investigations were carried out on rhizome of *Z. cassumunar* in order to determine the presence of alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavornoids, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins and terpenoids.

Preparation of various crude extracts from rhizome of Zingiber cassumunar Roxb. (Meik-tha-lin-oot)

The various crude extracts like PE, EtOH, EtOAc and H_2O extracts from the dried powder of rhizome of MTLO were prepared by using successive solvent extraction method.

Isolation of some organic constituents from EtOAc crude extract of rhizome of Zingiber cassumunar Roxb. (Meik-tha-lin-oot)

The EtOAc extract (8.74 g) of rhizome of MTLO was separated by silica gel column chromatography with PE:EtOAc gradient elution (9:1 and 4:1) to give one terpenoid compound: (0.00317%, amorphous orange crystals, M.pt=183°C). Then the isolated compound was characterized by melting points, R_f values, solubilities and some chemical tests such as treating with 5% H_2SO_4, vanillin-H_2SO_4, anisaldehyde-H_2SO_4, Liebermann-Burchard reagent on TLC chromatogram followed by treating with 1% $FeCl_3$ solution.

Screening of antimalarial activity

In vitro antimalarial activities of isolated compound, ethanol and watery extracts of rhizome of MTLO against *P. falciparum* were screened at the Parasitology Research Division in Department of Medical Research, Yangon.

Procedure for in vitro drug sensitivity test

Thick and thin blood films were prepared from three-hundred and twenty clinically-suspected malarial patients in Shwe Kyin who had fever within last one week and associated with chills, rigor, aches and pain. Twenty *P. falciparum* positive patients were selected for criteria matched blood sample. Informed consents were taken from all subjects. *In vitro* drug sensitivity test was performed following the micro-technique of *in vitro* micro test (mark III).[8] Blood 0.5 ml was collected with 1 ml disposable syringe from each of *P. falciparum* infected patients.

The blood sample was added to 4.5 ml of RPMI 1640 liquid medium in a sterile tube. Row (A) wells of the plates filled with patient's blood were used as control with the absence of drug. Dosing of the test sample were done (40, 80, 160, 320, 640, 1280, 2560 μg/ml, respectively) starting from the well (B) and then following an increasing order of concentration of test fraction up to well (H) for 95% ethanol extract of MTLO. Similarly, dosing of the test sample was done (46.88, 93.75, 187.5, 375.0, 750.0, 1500.0, 3000.0 μg/ml, respectively) starting from the well (B) and then following an increasing order of concentration of test fraction up to well (H) for watery extract of MTLO. In addition, dosing of the test sample was done (20, 40, 80, 160, 320, 640, 1280 μg/ml, respectively) starting from the well (B) and then following an increasing order of concentration of test fraction up to well (H) for isolated compound of MTLO. The *in vitro* chloroquine result of Mu Mu Sein Myint, *et al*,[9] was used as a standard drug. All well of each column were filled with 50 μl of the blood; medium ratio (1:9) using the eppendorf pipette with a disposable sterile tip. A new sterile disposable tip was always changed for every new row and the same way of blood: medium addition into each well was made. The microliter plate was covered with lid and shaken gently so that the test sample deposit in the well was completely dissolved. The test plate was placed in a candle jar and incubated at 37.3°C for 24-30 hours. After incubation, the contents of the test wells were harvested by removing the supernatant with an eppendorf pipette, and the series of thick film were made from deposited red blood cells on the flat bottom of the wells. The resultant thick films were carefully air-dried for 24-28 hours before staining, and they were stained for 45 minutes in 2% Giemsa stain.

The assessment was made by counting the number of schizonts against 200 asexual parasites in each film. The number of schizonts in the control was taken as 100 percent baseline for the assessment of schizont maturation in the various drug wells. Blood sample with a schizont maturation less than 10% in the control wells was not used for evaluation. The effective dose value was calculated using Wernsdorfer (1995) software.

RESULTS AND DISCUSSION

Preliminary phytochemical investigations

In order to find out the types of phytochemical constituents in the samples, the preliminary phytochemical examinations were firstly carried out according to the standard methods. From these experiments, α-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch and terpenoids were observed in rhizomes of the selected medicinal plant.

Preparation of crude extracts

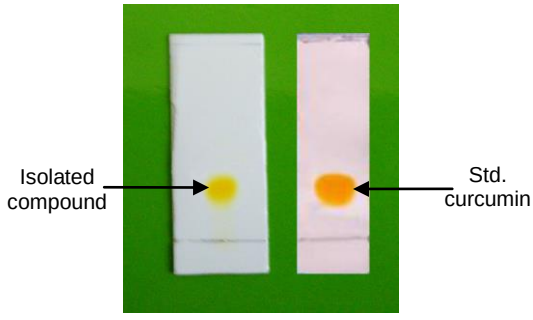
In this experiment, it was found that the non-polar constituents (PE extract, 7.11%), polar extract (EtOH extract, 3.44% and H_2O extract, 1.44%) and moderately polar extract (EtOAc extract, 1.64%) were obtained.

Characterization of some phytochemical constituents

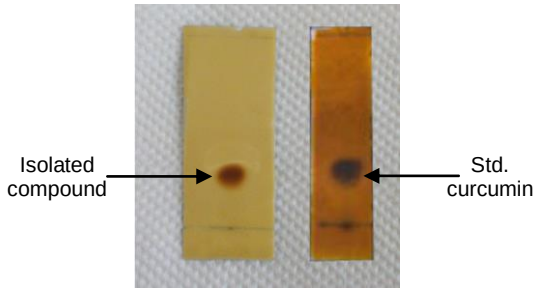
Some physic-chemical characteristics such as physical state, R_f value, melting point, solubility and the reactions with 1% $FeCl_3$, anisaldehyde, vanillin, 5%H_2SO_4 and Liebermann-Burchard reagents were compared with those of standard curcumin. From these results, it was investigated that some physico-chemical characteristics of the isolated compound were identical with that of standard curcumin (Figure 2). Therefore, it could be deduced that the isolated compound is assigned as curcumin.

Identification of isolated compound

UV spectral data - 423, 470 nm: FTIR spectral data - 3404, 3070, 2955, 2922, 2850, 1624, 1602, 1587, 1560, 1516, 1500, 1465, 1425, 1298, 1265, 1211, 1166, 1139, 1126, 1035, 962, 839, 804

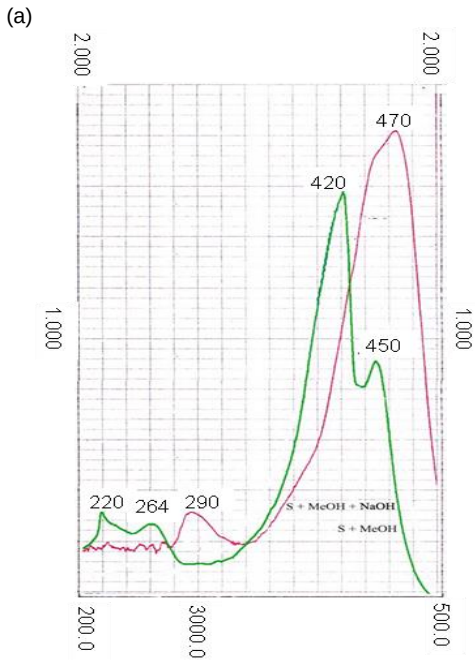


2(a) Under visible light
R_f = 0.45
Solvent system=PE: EtOAc
1:1 (v/v)



2(b) After treated with 1% $FeCl_3$
R_f =0.45

Fig. 2. TLC chromatograms of isolated compound and standard curcumin



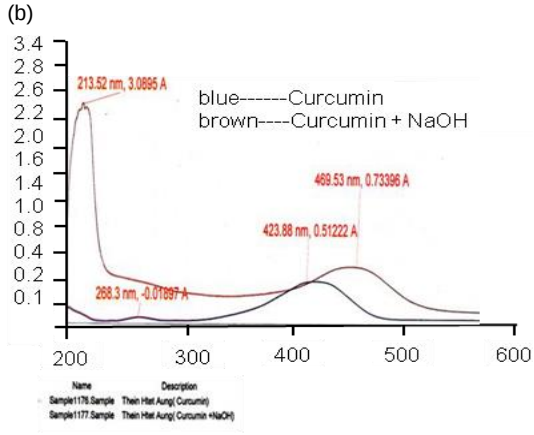


Fig. 3. UV spectrum of standard curcumin (a) and (b) isolated curcumin

It was investigated that the maximum absorption wavelength (423, 470) nm of the isolated compound (Fig. 3b) was found to be nearly the same as with the reported λ_{max} (420, 470) nm of curcumin[10, 11] (Fig. 3). Similarly, the FTIR spectral results of the isolated compound were compared with that of the standard curcumin (Fig. 4).

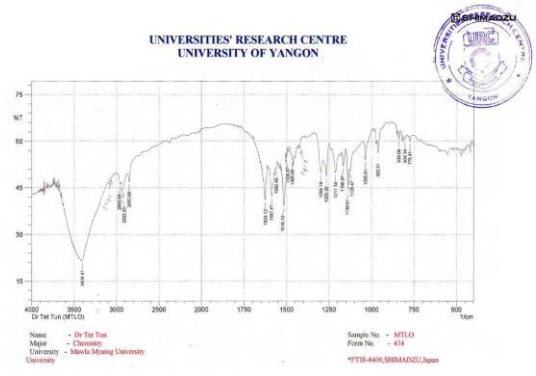
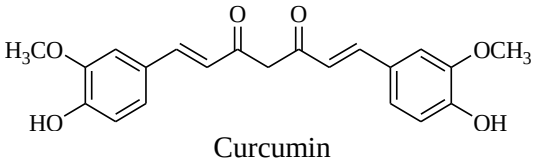


Fig. 4. FTIR spectrum of isolated curcumin

From this comparison, it was found that the significant frequencies of the important functional groups of the isolated compound such as phenolic group, OCH_3 group and C=O with conjugated double bond systems were nearly the same as that of the standard curcumin. Therefore, according to UV and FTIR spectral results, it can be inferred that the isolated compound is identified as curcumin.



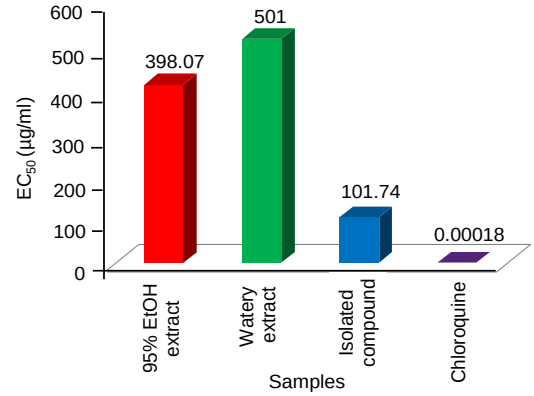
Curcumin

Investigation of antimalarial activity

In this experiment, the malaria culture was firstly prepared. Schizont maturation inhibition (SMI%) was determined by using different concentrations of 95% ethanol extract. Similarly, SMI% for watery extract and isolated compound (curcumin) was also determined by using their respective concentrations. From this experiment, it was investigated that the percentage of schizont maturation inhibitions (SMI%) were found to increase with increasing concentrations of 95% ethanol and watery extracts and isolated compound. In comparison of three samples, the ability of the isolated compound (curcumin) to inhibit the development of schizonts was greater than those of the remaining two samples (95% ethanol and watery extracts).

After preparing of malaria culture, the antimalarial activity of isolated compound, ethanol and watery extracts was screened by using assessment method. The effective concentrations (EC) were calculated by using Wernsdorfer (1995) software. The values of EC_{50}, EC_{95} and EC_{99} were found to be 101.74 µg/ml (between 81.57 µg/ml and 126.89 µg/ml 95% confidence interval), 681.99 µg/ml (between 453.44 µg/ml and 1025.75 µg/ml 95% confidence interval) and 1499.93 µg/ml (between 877.22 µg/ml and 2564.66 µg/ml 95% confidence interval), respectively. The lower the EC_{50} value, the higher the activity.

According to these results, it was found that the isolated compound (curcumin) obtained from EtOAc crude extract of MTLO exhibited the moderate antimalarial activity since the EC_{50} value, 101.74 µg/ml was within the range of 20 µg/ml and 200 µg/ml. The comparative results of chloroquine, isolated compound, ethanol and watery extracts of MTLO were illustrated in Fig. 5.



***<20 µg/ml=high activity, **>20 µg/ml and <200 µg/ml=moderate activity, *>200 µg/ml=no activity

Fig. 5. The anti-malarial activiting of isolated compound, ethanol and watery extracts of MTLO against *P. falciparum* isolates compared with standard chloroquine

Although the relationship between the structure-activity correlation of curcumin and its antimalarial activity had not known yet, it may be estimated that the structure containing carbonyl group, phenol group, methoxy group and conjugated double bonds may support the ability of its antimalarial activity by one way or another. In context of malaria, curcumin has been shown to possess moderate antimalarial activity with IC_{50} value of 5-18 µM.[12, 13, 14]

Conclusion

From the preliminary phytochemical investigations, it was found that α-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch and terpenoids were present. Curcumin (0.00137% yield, orange amorphous crystal, M.pt=183°C) was isolated from EtOAc crude extract of *Z. cassumunar* Roxb. (Meik-tha-lin-oot) by using thin layer and column chromatographic separation techniques. Isolated compound showed the moderate antimalarial activity with 101.74 µg/ml of EC_{50} value. Therefore, curcumin has moderate antimalarial activity

according to literature value (EC_{50} <200 μg/ml)[9] and may be used as the antimalarial agent.

Competing interest

There authors declare that they have no competing interest

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