Study on Preliminary Phytochemical Investigation and Antibacterial Activity of *Curcuma Longa* L. (Na-Nwin)

Ei Ei Khaing

Abstract

The rhizome of *Curcuma longa* L. (Na-nwin) used in household remedy for medicinal purposes, colouring materials, insecticide and fungicide was chosen for present study. In Myanmar, gastrointestinal (GI) disorders are one of the major health problems especially in Ayeyarwady Region which local people face dysentery and diarrhoea concerned with the flooded problem. In fact, a localized problem was related to public health. Therefore, the present research designated to explore the promising Myanmar medicinal plant in the use of traditional drug for dysentery and diarrhoea. The aim of the study is to examine the phytoconstituents and to screen the antibacterial activity of three crude drugs from the rhizome of *Curcuma longa* L. (Na-nwin). Preliminary phytochemical investigation of rhizome of Na-nwin was performed by using test tube method. Some phytochemical constituents such as α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponin glycosides, steroids, tannins and terpenoid compounds were found to be present whereas alkaloids, cyanogenic glycosides and reducing sugar were absent. Then, three crude extracts of the sample were prepared by using various solvents: petroleum ether, 96 % ethanol and 50 % ethanol. *In vitro* antibacterial activity of three crude extracts was investigated against three bacterial strains: *Shigella dysentery*, *Escherichia coli* and *Pseudomonas aeruginosa* by using agar disc diffusion method. It was found that the polar and non-polar extracts of *Curcuma longa* L. showed potent antibacterial activity on three tested bacteria with the remarkable zone diameters (18-24) mm in comparison with cifran used as standard.

Keywords: *Curcuma longa*, Na-nwin, phytoconstituents, crude extracts, antibacterial activity

Introduction

Diarrhoea and dysentery are important health problems in worldwide especially developing countries. So the Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in combating six major types of diseases: namely; malaria, tuberculosis, diarrhoea, dysentery, diabetes and hypertension. Diarrhoea is the host response to infection of the gastrointestinal tract by a variety of viruses, bacteria and parasites. There are three types of diarrhoea, namely acute diarrhoea, persistent diarrhoea and chronic diarrhoea. Acute diarrhoea is usually defined as the passage of 3 (or) more liquid motions within 7 days. Persistent diarrhoea has a usually long duration, more than 2 weeks, but usually less than 2 weeks duration. Chronic diarrhoea lasts for more than three weeks (Khan, 2001).

Dysentery is an inflammatory disorder of the lower intestinal tract, usually caused by bacteria, parasite, or protozoa infection and resulting in pain, fever, and severe diarrhoea, often accompanied by the passage of blood and mucous. Dysentery is caused by an *Amoeba* or *Bacillus* that infects the colon (Boyd and Marr, 1980).

The study of traditional medicinal plants and their therapeutics play a very important role in health care system of Myanmar because 70 % of its population is in the rural area and they have been using traditional medicine for centuries (Dahanukar *et.al*, 2000).

In this study, Myanmar medicinal plant, *Curcuma longa* L. (Na-nwin) was selected to find out of active principle for the treatment of dysentery and diarrhoea. *C. longa* distinctly showed anti-oxidant activity and also had a prominent antibacterial activity. Therefore, it is

Associate Professor, Dr, Department of Chemistry, Hinthada University

very popular for the treatment of related to the gastrointestinal (GI) tract infection. In fact it could be able to be antioxidant and precious pharmacological activity in human body because curcumin, a yellow pigment compound mainly contained in it (Wagner and Bladt, 2005). Na-nwin is composed of health benefiting essential oils besides a polyphenolic compound, curcumin, gives bitter flavor, spicy pungent and yellow pigment character to the rhizome of *C. longa*. Therefore, it is medicinally used in various ailments, fever, cough, dysentery, diarrhoea, cancer, bleeding, foul ulcer, mosquito coil, urinary diseases, liver disorder, ringworm and other skin diseases. Nasal catarrh and hysterical fits may be remedied by inhaling fumes of burning Na-nwin. A paste of Na-nwin and quick lime is an excellent dressing for sprains and inflammatory joints. *C. longa* has pharmacological activities such as antioxidant, antibacterial, antifungal, insecticidal, antitumor and anti-inflammatory (Upadhyaya, 1998). Na-nwin is a very important spice in India for religious such as Hindu ceremony like a wedding besides used as cosmetic such facial mask for anti-aging process. As the bright yellow colour of Na-nwin, it makes food additives with important ingredient of masalas and curry powders in kitchen. This colour is used for colouring butter, cheese, pickles and other food stuff as well as for dyeing cotton, silk and woollen materials.

In Myanmar, *C. longa* is used as the house hold remedy in treating diarrhoea, dysentery, fever, cough, indigestion, stomachaches and prevention of mosquito. Therefore, some phytoconstituents known as efficacious medicine were evaluated and antibacterial activity investigation on three crude extracts (PE, 96 % EtOH, 50 % EtOH) from the rhizome of *C. longa* were carried out by using agar disc diffusion method in this study.

Botanical Aspects of *Curcuma longa* L.

Scientific name : *Curcuma longa* L.

English name : Turmeric

Myanmar name : Na-nwin

Family : Zingiberaceae

Rhizome (part used) : Rhizome is tuberous with a rough, segmented skin and yellowish-brown with a dull orange interior that looks bright yellow when powdered (Kress, *et al*., 2003).

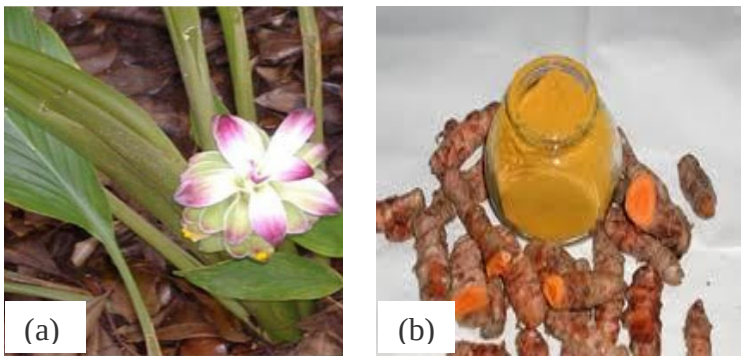


Figure (1). (a) Plant of *Curcuma longa* L.
(b) Rhizome of *Curcuma longa* L.

Distribution

Curcuma longa L. (Na-nwin) shown in figure 1 (a) and (b) is a native of Southern or South-east Asia. It has been grown in India, China, Hawaii and Easter Island. In Myanmar, it can be occurred in throughout country especially in Hinthada Township, Ayeyarwady Region planted for economy because of its coloring materials and medicinal properties (Upadhyaya, 1998).

Chemical Constituents

Curcuma longa contains an essential oil, turmerone, zingiberene and conjugated diarylheptanoids (1,7- diaryl-hepta-1-6-diene-3,5-diones, e.g. curcumin) (Upadhyaya, 1998).

Materials and Methods

Plant Material

The rhizome sample of *C. longa* L. (Na-nwin) was collected from Zalun Township, Hinthada District, Ayeyarwady Region. It was identified by a botanist from Department of Botany, Hinthada University. The rhizome of *C. longa* was washed, cleaned and dried at room temperature. Then the dried sample was powdered and stored in air- tight container.

Instruments

Petridishes, sterile cotton swab (Puritan, USA), paper disc with a 6 mm diameter (Toyo No. 26, Japan), incubator and autoclave at National Health of Laboratory (NHL), Yangon

Chemicals

Phytochemicals: sulphuric acid, hydrochloric acid, sodium hydroxide, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Ninhydrin reagent, gelatin solution, $FeCl_3$ solution, Benedict's solution, Extraction: Petroleum ether (PE), ethanol (EtOH), (non-polar and polar solvent) and Bacteria: trypticase soy agar

Preliminary Phytochemical Tests of Rhizome of *C. longa* by Using Test Tube Method

Preliminary phytochemical investigation was carried out on powdered, dried sample of rhizome of *C. longa* with a view to determine the presence or absence of alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, flavonoids, glycosides, phenolic compounds, reducing sugar, saponin glycosides, steroids, tannins and terpenoids.

Preparation of Extracts from Rhizome of *C. longa* by Cold Extraction Method

The air-dried powder (500 g) was individually cold extracted with (1000 mL) of solvents; petroleum ether (60-80 $^{\circ}$C), 96 % ethanol and 50 % ethanol for 7 days and then filtered. The filtrate was evaporated to dryness at normal pressure on a water bath and desiccated. The yield % of petroleum ether (PE), 96 % ethanol (EtOH) and 50 % ethanol extract were determined.

In vitro Studies on the Antibacterial Activity of Rhizome of *C. longa* by Agar Disc Diffusion Method

Screening of antibacterial activity of crude extracts against three tested bacterial strains

Agar disc diffusion method was used for the detection of antibacterial activity for three crude extracts from *C. longa* rhizome. The test procedure was as follows: the extracts (1 g each) were dissolved in 1 mL of their respective solvents; petroleum ether, 96 % ethanol and 50 % ethanol, and introduced into sterile petridishes for testing 3 cultural bacterial strains from clinical sources, National Health Laboratory (NHL), Yangon; related to acute diarrhoea (cholera), dysentery, abscess in gastrointestinal (GI) tract.

The discs having 6 mm diameter each with 20 µg extract/disc were allowed to dry at 42 $^{\circ}$ C in incubator. The bacterial suspension from trypticase soy broth was streaked evenly into three places on the surface of the trypticase soy agar plates with sterile cotton swab (Puritan, USA). After the inoculums had dried for 5 min, the dried disc impregnated with extracts were placed on the agar with flamed forceps and gently pressed down to ensure proper contact. A disc impregnated with ethanol solvent only was used as control besides antibiotics cifran treated a variety of bacterial infections such as bronchitis, pneumonia and GI problems was also used as standard for this study. After overnight incubation at 37 $^{\circ}$C, the zones of inhibition diameter including 6 mm discs were measured.

Results and Discussion

Preliminary Phytochemical Examination of Rhizome of *C. longa*

In this research work, rhizome of *C. longa* was collected from Zalon Township, Hinthada District, Ayeyarwady Region and then made the powdered samples, and stored in air-tight bottles for phytochemical investigations. Phytochemical screening serves as an initial step in expanding the knowledge about plant constituents and interest of phytochemists trying to recover new sources of phytochemical and to devise schemes for isolation and structural elucidation of biologically active compounds. For this reason, the phytochemical test was carried out on rhizome of *C. longa* and it was found that rhizome of *C. longa* consists of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponin glycosides, steroids, tannins and terpenoid compounds. However, alkaloids, cyanogenic glycosides and reducing sugar were not found in it. The obtained results were summarized in Table 1.

Extraction of *C. longa* Rhizome

The dried rhizome powder collected from Zalon Township, Hinthada District, Ayeyarwady Region was extracted with various non-polar and polar solvents and the yield % of petroleum ether extract (1.50 %), 96 % ethanol extract (4.50 %) and 50 % ethanol extract (6.20 %) respectively were obtained. It was found that polar compounds such as popular for antioxidant polyphenolic compounds more contained in Na-nwin powder than that of non-polar compounds. Therefore Na-nwin especially used as daily curry powder possesses very precious medicinal properties.

Screening of Antibacterial Activity of Crude Extracts of *C. longa*

Screening of antibacterial activity of the three crude extracts has been done by agar disc diffusion method at NHL. Agar disc diffusion method is based on the zone diameter in millimeter (mm) of agar disc. Larger the zone diameter is the more activity on the tested bacteria. The inhibition zone diameters of extracts tested with 3 species of bacteria took out

the patients suffering from diarrhoea, dysentery and abscess in GI tract to know how the selected plant effect on the bacteria from the clinical sources shown in Table 2, Figure 2 and Figure 3. The 3 tested bacteria comprised of the Gram - negative bacteria: *Shigella dysentery*, E*scherichia coli* and *Pseudomonas aeruginosa*. Out of the three crude extracts of *C. longa* rhizome, PE extract remarkable showed against all tested bacteria with the inhibition zone diameter range between (20-24) mm although cifran (standard) resulted with the range of inhibition zone diameter (20-21) mm against 3 strains: *Shigella dysentery*, E*scherichia coli* and *Pseudomonas aeruginosa*. The results could be assigned that it may be caused by the sensitivity of synergistic effect or non-polar compounds in the most active petroleum ether extract. The different ethanol extracts (96 % and 50 %) exhibited antibacterial activity on all tested bacteria strains cultured from patients suffered from diarrhoea, dysentery and abscess in GI tract. In fact both ethanol extracts were effective on all tested bacteria as near as cifran (std.) stated range of inhibition (20-21) mm because it was observed that 96 % ethanol extract showed activity with the range of inhibition zone diameter (18-21) mm as well as inhibition zone diameter of 50 % ethanol extract was (19-20) mm. In general, *C. longa* rhizome possess affective bactericidal activity and it could be recommended that the plant's antibacterial substances appear to be inhibition to Gram-negative bacteria type as the results of inhibition zone diameter. The overall results of throughout the antibacterial screening could be evaluated and assigned that petroleum extract displayed more potent than the different crude extracts of ethanol solvent obtained from *C. longa* rhizome in comparison with cifran (std.). Therefore PE extract from *C. longa* rhizome would be more effective for the treatment in diarrhoea, dysentery and abscess in GI tract. It has antibacterial action against E*scherichia coli* responsible for diarrhoea, *Shigella dysentery* responsible for dysentery and *Pseudomonas aeruginosa* responsible for abscess occurred in gastrointestinal (GI) tract.

Table (1). Results of Preliminary Phytochemical Examination of Rhizome of *C. longa*

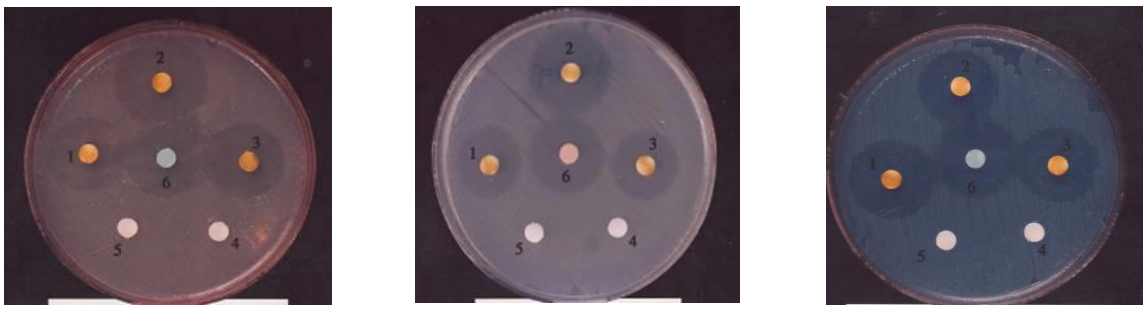
No	Test	Extract	Reagent Used	Observation	Remark
1	Alkaloids	1 % HCl	Mayer's reagent Dragendorff's reagent Wagner's reagent	No white ppt. No orange ppt. No reddish brown ppt.	(-) (-) (-)
2	α-amino acids	D/W	Ninhydrin reagent	Pink spot	(+)
3	Carbohydrates	D/W	10% α-naphthol and 1 mL of Conc: H_2SO_4	Red ring	(+)
4	Cyanogenic Glycosides	D/W	Sodium picrate and conc. H_2SO_4	No brick-red colour	(-)
5	Flavonoids	80 % EtOH	1 % HCl and Mg Turning	Red colour solution	(+)
6	Glycosides	D/W	10 % lead acetate solution	White ppt.	(+)
7	Phenolic Compounds	D/W	$FeCl_3$ solution	Deep blue colour solution	(+)
8	Reducing Sugar	Dil H_2SO_4 and NaOH	Benedict's solution	No red colour solution	(-)
9	Saponin Glycosides	D/W	Distilled water	Marked frothing	(+)
10	Steriods	Toluene	Acetic anhydride and Conc: H_2SO_4	Green colour solution	(+)
11	Tannins	D/W	2 % NaCl and 1 % gelatin	White ppt.	(+)
12	Terpenoids	EtOH	Acetic anhydride and Conc: H_2SO_4	Red colour solution	(+)

(+) = presence (-) = absence (Ei Ei Khaing, 2011)

Table (2). Results of Antibacterial Activity of Three Crude Extracts of *C. longa* on 3 Species of Bacteria

No.	Test samples	Inhibition zone diameter (mm) against tested bacteria		
		Shigella dysentery Gram (-)	*Escherichia coli* Gram (-)	*Pseudomonas aeruginosa* Gram (-)
1	96 % EtOH extract	20	18	21
2	P.E extract	24	23	20
3	50% EtOH extract	20	20	19
4	EtOH solvent (control)	-	-	-
5	Blank	-	-	-
6	Cifran (standard)	21	21	20

(-) = no activity, Disc diameter = 6 mm (Ei Ei Khaing, 2011)



Shigella dysentery *Escherichia coli* *Pseudomonas aeruginosa*

Clockwise position

1 = 96 % EtOH extract 4 = EtOH solvent (control)

2 = PE extract 5 = Blank

3 = 50 % EtOH extract 6 = Cifran (standard)

Figure (2). Antibacterial activity of PE, 96 % EtOH and 50 % EtOH crude extracts of *C. longa* (Na-nwin)

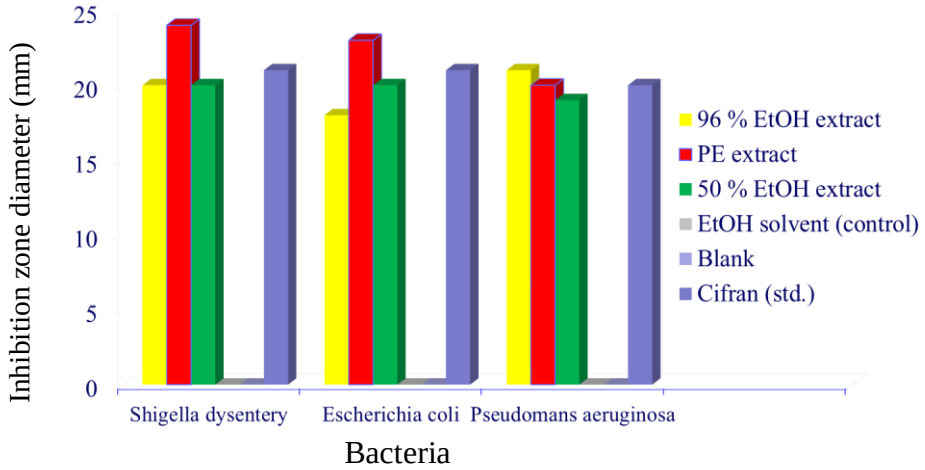


Figure (3). Antibacterial activity of PE, 96 % EtOH and 50 % EtOH crude extracts of *C. longa* (Na-nwin) against three tested bacteria

Conclusion

The preliminary phytochemical investigation was carried out on powdered, dried rhizome of *Curcuma longa* L. (Na-nwin) with a view to determine the absence of alkaloids, cyanogenic glycosides and reducing sugar. However α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponin glycosides, steroids, tannins and terpenoid compounds were found in the rhizome of Na-nwin. Thus it may be regarded that organic constituents such as flovonoids, steroids and phenolic compounds are used medicinally in very small quantities. Due to the absence of very poisonous compounds, cyanogenic glycosides, Na-nwin may not have detrimental effect on human health but it should be taken a small amount in diet to avoid over dose.

From the rhizome of *C. longa*, non-polar and polar three crude extracts: PE extract (1.50 %), 96 % EtOH extract (4.50 %) and 50 % EtOH extract (6.20 %) were obtained and screened the antibacterial activity against 3 tested bacteria by agar disc diffusion method. Among the three crude extracts of Na-nwin, only PE extract showed the most potent antibacterial activity with the related larger zone diameter (20-24) mm on 3 bacterial strains: *Shigella dysentery, Escherichia coli* and *Pseudomonas aeruginosa* responsible for diarrhoea, dysentery and abscess in GI tract. Among the extracts of different ethanol solvent (%), 96 % ethanol extract showed the range of inhibition zone diameter (18-21) mm and the next, 50 % ethanol extract resulted with the range between (19-20) mm by comparison with inhibition zone diameter of standard cifran ranged between (20-21) mm. In addition, polar and non-polar extracts of rhizome of *C. longa* (Na-nwin) showed *in vitro* antibacterial sensitivity against some pathogenic bacteria causing dysentery, diarrhoea and abscess in GI tract, etc.

In fact that the selected Myanmar medicinal plant (Na-nwin) should be used in a herbal formulation for the treatment of diarrhoea, dysentery, abscess in GI tract and preventing from pathogenic bacteria. Since very poisonous cyanogenic glycosides were not found in Na-nwin rhizome benefit to human health, it could be advocated to be safely and widely used in food industry. Therefore, *C. longa* (Na-nwin) should be cultivated not only for the commercial production as local needs but also for the product of export.

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