Introduction

*Curcuma longa* L. (Zingiberaceae) or turmeric is a perennial herb widely cultivated in tropical regions of Asia. Its rhizome is extensively used as coloring matters in dyeing, cosmetics, and food seasonings. In medicine, turmeric was claimed to be stomachic, stimulant, carminative, hematic or styptic in all kinds of hemorrhages. It had also been used as a remedy for a certain type of jaundice and other liver trouble. It was applied externally to treat minor wounds and certain skin eruptions. A decoction afforded relief for a
burning sensation in eye disease.\(^{(2,3)}\) It was considered to be very good for irregular menstruation.\(^{(3)}\)

It also promoted circulation, dissolves blood clots\(^{(4)}\) and was prescribed as a remedy for abdominal, chest, and back pains\(^{(5)}\). Turmeric rhizome was reported to contain essential oil, (4.5%), an orange yellow pigment (4.8%), arabinose (1.1%), fructose (12%), and glucose (28%). The essential oil was found to contain \(\alpha\)-and \(\beta\)-pinene, camphene, limonene, terpinene, \(d\)-\(a\)-phellandrene, \(d\)-sabinene, cineole, borneol, caryophyllene, curcumene, zingiberene, linalool, turmerone, \(ar\)-turmerone, isoborneol, camphor, eugenol, curdione, curzermenone\(^{(5-4)}\). The three major constituents of turmeric were found to be curcumin\(^{(2)}\) which formed the most important fraction and the other two being derivatives of curcumin, i.e. desmethoxycurcumin\(^{(3)}\) and bisdesmethoxy curcumin\(^{(4)}\). The chemical structures of curcumin and its derivatives have been reported\(^{(9)}\).

![Fig. 1 Structures of some major constituents of Curcuma longa L.](attachment:image.png)

**Fig. 1 Structures of some major constituents of Curcuma longa L.**

\(ar\)-turmerone (1), curcumle (2), Desmethoxycurcumin (3) and bisdesmethoxycurcumin (4)

Numerous works on pharmacological study of firemeric have been reported. Yegnanarayan et al.\(^{(1)}\) reported the antiinflammatory activity of *Curcuma longa* L. on acute inflammation using acute carragenan-induced rat paw edema model, it was found that among various extracts, the water extract was the most active with an ED\(_{50}\) of 4.7 mg/kg compared to 40.7 mg/kg for Petroleum ether extract and 309 mg/kg for ethanol extract. Antiinflammatory activity of curcumin has also been studied\(^{(10)}\). Systematic investigations on antiinflammatory activity of the rhizome of *Curcuma longa* L. have been carried out and the results indicated that curcumin was the major compound responsible for antiinflammatory activity\(^{(10)}\). A wound healing property of turmeric powder was also...
reported, application of turmeric powder over septic as well as aseptic wounds accelerated the healing process to the extent of 23-24%\(^{(10)}\). The effects of turmeric on stomach have also been studied. It was found that curcuma powder increased the mucin content of gastric juice in rabbit which would be beneficial in protecting the gastric mucosa against irritants\(^{(10)}\). Controversial data on antipeptic ulcer activity of curcumin, the major component of Curcuma longa L., have been reported. Sinha et al. reported the antiulcerogenic activity of curcumin\(^{(11)}\) whereas Bhatia et al. indicates that curcumin did not show any protective action against histamine-induced gastric ulceration in guinea pigs\(^{(12)}\). Furthermore, even an ulcerogenic effect of curcumin at high dose was observed\(^{(13)}\). A recent review on the pharmacological studies of Curcuma longa L. has been published\(^{(14)}\).

Although turmeric has been reported to be effective against peptic ulcer,\(^{(15)}\) however, the active components have not been identified. This work attempts to isolate and characterize such active principles of Curcuma longa L.

**Materials and Methods**

Fresh rhizomes of Curcuma longa L. were obtained from Botanical section, Division of medicinal Plant Research and Development, Department of Medical Sciences. The sample was cleaned, sliced and dried in the hot air oven at 50°C. The solvents, obtained commercially, were distilled before used. Column chromatography was carried out on a silica gel column (70-230 mesh, E. Merck). Mass spectrum was obtained from a JMS-D100 Jeol Mass spectrometer. High resolution mass spectrum was performed on a JMS DX-705L Jeol Mass spectrometer, optical rotation on a Jasco DIP-181 digital polarimeter and infrared spectra from a Jasco A-302 Infrared spectrophotometer. Preparative HPLC was performed on a Waters 820 Chromatography data station, a Waters 510 HPLC pump, a Waters 710 WISP system processor and a Waters 490 programmable multiwavelength detector; a 7.8 mm x 30 cm bondapak C\(_18\) was used with a flow rate of 2 ml/min and a UV detection of 235 nm; 9:1 acetonitrile:water was used as a mobile phase. Nuclear Magnetic Resonance spectra were obtained from a 60 MHz EM-360L Varian NMR spectrometer or a 500 MHz JNM-GX500 Jeol NMR spectrometer and the chemical shifts were reported in parts per million downfield from tetramethylsilane.

**Isolation of Antipeptic ulcer principle**

Dried powder of Curcuma longa L. rhizome (1000 g) was extracted in a Soxhlet extractor with 95% ethanol ras 10 days. The ethanolic solution was concentrated on a rotary evaporator to provide 305.40 g of crude alcoholic extract. The whole ethanol extract was repeatedly shaken with hexane and the layers were separated. The combined hexane and ethanol extracts were concentrated on the rotary evaporator to provide 98.44 g and 209.88 g of hexane soluble and hexane non-soluble fraction, respectively. Both fractions were
subjected to the antipeptic ulcer test. It was found that the hexane soluble fraction was effective against HCl-induced peptic ulcer while the hexane non-soluble fraction was inactive. Isolation of active constituents was affected by placing the hexane soluble fraction (10 g) on a silica gel column (300 g) eluted with 10-30% ethylacetate/hexane to provide 4 column fractions: CD-1 (0.86 g), CD-2 (4.00 g), CD-3 (0.63 g) and CD-4 (0.65 g). All four fractions were again tested for the antipeptic ulcer activity. It was found that only fraction CD-2 showed antipeptic ulcer activity.

Further purification to remove traces of impurities was performed by multiple column chromatography followed by preparative HPLC to provide compound CD-2 as a light yellow liquid bp, 156-160°C lit bp 159-160°C. [α]D = -66° CHCl3 (c 0.546). MS m/z (relative intensity) 216 (M +, 28), 201 (12), 149(16), 119(70) and 83(100). IR (thin film) 2960, 2940, 1685, 1620, 1520, 1440, 1380, 1110, 1050 and 820 cm⁻¹. ¹H NMR (60 MHz) (CDCl3) δ 7.00 (s, 4H, Ar-H), 5.87 (broad s, 1H, C-CH,), 3.23 (m, 1H, Ar-CH), 2.60 (m, 2H, CO-CH,), 2.26 (s, 1H, Ar-CH), 2.07 (broad s, 3H, C=C-CH,), 1.80 (broad s, 3H, C=C-CH,) and 1.17 ppm (d, 3H, J=6.93 Hz, Ar-CH-CH,).
Results and Discussion

CD-2 was isolated in high yield (-4% calculated from powdered drug). Spectroscopic characterization of the antipeptic ulcer principle clearly indicated that CD-2 was ar-turmerone (1), one of the major components of the essential oil of *Curcuma longa* L. Mass spectrum showed the parent mass (M') of 216. High resolution mass spectrum calculated for C_{20}H_{16}O: 216.3224; Found: 216.1525. NMR spectra of the compound strongly support this conclusion (Fig.2). The aromatic protons showed a downfield singlet of a proton at 7.00 ppm. A broad singlet of one proton at 5.87 ppm belongs to the vinylic proton. The benzylic proton, was splitted by the adjacent methyl and methylene protons, to give a multiplet at 3.23 ppm. The four methyl groups appeared as three singlets and one doublet. The aromatic methyl group resonated at 2.26 ppm to give a singlet. The benzylic methyl group, splitted by the vicinal methine proton, appeared as a doublet at 1.17 ppm. (J=6.93 Hz). Geminal dimethyl group gave two broad singlets at 2.07 and 1.80 ppm, the lower field signal belongs to the methyl group which was deshielded by the carbonyl group.

An interesting observation should be noted for the signal of the methylene protons which became nonequivalent when they are adjacent to an asymmetric center[11]. The 60 MHz spectrometer gave a partially resolved signal at 2.60 ppm due to geminal coupling and coupling with adjacent methine proton(Fig.3). However, from a higher frequency instrument, (500 MHz), an ABX system was observed for the methylene protons and the
Fig. 3 Methylene protons signal of CD-2 (-2.6 ppm) observed from (a) 60 MHz and (b) 500 MHz $^1$H NMR spectrometer.

Fig. 4 Double doublet signals of the methylene protons of CD-2 observed from 500 MHz $^1$H NMR Spectrometer.
benzylic proton. The methylene protons appeared as two double doublets at 2.60 and 2.71 ppm respectively. (Fig.4). The two methylene protons were equally splitted \( (J_{AB} = 15.51 \text{ Hz}) \) by each other followed by unequally splitting \( (J_{AX} = 6.27 \text{ and } J_{AA} = 8.25 \text{ Hz}) \) by the vicinal methine proton (Fig.4). This occurrence has been previously described by Silverstein, et al.\(^{(11)}\); however, their observation has not been in the very same molecule as reported here.

The isolation procedure reported in this paper intended for identifying of the antipeptic ulcer principle from Curcuma longa L. More readily methods for obtaining ar-turmerone was previously reported, both from the isolation and synthesis\(^{(12)}\). Further work is in progress to isolate a larger amount of this active principle to obtain more information of the compound on other fields involved, i.e. pharmacokinetics, toxicology, carcinogenicity, etc. in order to develop this antipeptic ulcer principle to a modern drug in the near future.

**Acknowledgements**

The author would like to thank Professor M.Isobe, Nagoya University, Nagoya, Japan, for kindly providing the mass spectrum, NMR spectrum and optical rotation; Mr. Theerawuth Pinthong, chief of Pharmacokinetic section, Division of Medicinal Plant Research and Development for providing the samples of turmeric.

**References**


15. The experiment was carried out at the Pharmacological section, Division of Medicinal Plant Research and Development, Department of Medical Sciences. The results of this experiment will be published separately.


