A study on the cholagogic effects of commercial turmeric and zedoary articles

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Abstract

The cholagogic effects of Japanese Haru-ukon (SU), Aki-ukon (AU), Ryukyu-gajutsu (RG), and Taiwanese commercial curcuma rhizome (CR), turmeric (CT) and zedoaria (ZR) were studied. The results show that all the *n*-hexane and ethanol extracts of these turmeric and zedoary articles administered in a dose of 2,000 mg/kg body weight, intraduodenally (id.) can increase bile secretion in the first 30-minute interval up to 2–2.5 times that before the administration in all test groups of rats and such cholagogic effect is of good continuance. This is especially noted in the ethanol extracts of SU (SU-A), AU (AU-A), CT (CT-A) and CR (CR-A) which are of higher potency and continuance in that they still maintain a cholagogic effect producing 2 times or nearly 2 times bile secretion 2.5 hours after their administration, or 1.5 times more or nearly 1.5 times bile secretion 5 hours after administration compared with the bile secretion before their administration in the rat.

Key words cholagogic effect, curcuma rhizome, curcuminoids, ethanol extract, *n*-hexane extract, turmeric, zedoaria.

Abbreviations AU, Aki-ukon ; AU-A, ethanol extract of AU ; AU-H, *n*-hexane extract of AU ; CR, Taiwanese commercial curcuma rhizome ; CR-A, ethanol extract of CR ; CR-H, *n*-hexane extract of CR ; CT, Taiwanese commercial turmeric ; CT-A, ethanol extract of CT ; CT-H, *n*-hexane extract of CT ; DDCM, *p,p'*-dihydroxydicinnamoylmethane ; HCFM, *p*-hydroxycinnamoylferuloylmethane ; id., intraduodenally ; RG, Ryukyu-gajutsu ; RG-A, ethanol extract of RG ; RG-H, *n*-hexane extract of RG ; SDC, sodium dehydrocholate ; SU, Haru-ukon ; SU-A, ethanol extract of SU ; SU-H, *n*-hexane extract of SU ; ZR, Taiwanese commercial zedoaria ; ZR-A, ethanol extract of ZR ; ZR-H, *n*-hexane extract of ZR.

Introduction

Curcuma rhizome, called "Ukon" in Japanese is the dried rhizome of *Curcuma longa* LINN. of the Zingiberaceae family. It is called "Jiang-huang (姜黃)" in China, whereas, turmeric, called "Haru-ukon" in Japanese, is the dried tuber of *C. aromatica* SALISB., which is known as "Yu-jin (郁金)" in China. "Yu-jin" and "Jiang-huang" look alike externally, and their naming is also very confusing in China. However, in Japan, "Ukon" is the only term used generally to refer to the two items. Zedoaria, called "Gajutsu" herb is the dried rhizome of *C. zedoaria* ROSCOE, which is called "E-zhu (莪朮)" in China.[1-4]

Curcuma rhizome or turmeric has been used as an aromatic stomachic commonly incorporated in various formulas. It is also used as a cholagogue for treating hepatitis, cholangitis, cholelithiasis and jaundice. For other purposes, the herb also has been used as a styptic and emmenagogue for hematemesis, hematuria, dysmenorrhea, costalgia and abdomenache, and also for omarthritis and inappetence. Zedoaria is also used as an aromatic stomachic, excitant, carminative, analgesic and emmenagogue indicated for dyspepsia, colics and dysmenorrhea.[5-7] In China

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the herb's "blood-stasis destroying" effect is also applied to the treatment of various intraperitoneal neoplasms, menalgia and lower abdominal neoplasms.[5] In other words, the chief pharmacological activities with curcuma rhizome, turmeric and zedoaria include digestive effect, stomachic effect and choleretic effect, *etc.*.[1, 2, 5-7]

The present study was undertaken with an attempt to examine and compare the cholagogic activity of different commercial turmeric and zedoary articles obtained from the markets in Japan and Taiwan. At the same time, the assays of the main color constituents, i.e. curcuminoids including curcumin, *p*-hydroxycinnamoylferuloylmethane (HCFM) and *p,p'*-dihydroxydicinnamoylmethane (DDCM) were also carried out.

Materials and Methods

Materials : Three Japanese articles, namely Haru-ukon (SU), Aki-ukon (AU) and Ryukyu-gajutsu (RG) used in this experiment were supplied by Nihon Ukon Sangyo Co., Ltd., Ohmuta, Fukuoka, Japan, and three Taiwanese commercial articles, namely curcuma rhizome (CR), turmeric (CT) and zedoaria (ZR) were purchased from Lao Cheng Chi Drug Store in Taipei, Taiwan. Curcumin, sodium dehydrocholate (SDC) and acenaphthene were purchased from Sigma Chemical Co., St. Louis, Mo., U.S.A. HCFM and DDCM were isolated from CR and confirmed by comparing their IR, UV, HNMR and MS with those from the literature.[8] Purity checking and peak identifying for all indexing standards and test samples were done with a photodiode array detector. Male Long-Evans rats (outbred), about 7 weeks old, purchased from Laboratory Animal Center, College of Medicine, National Taiwan University in Taipei were further raised after purchase and observed for a week before being selected and used for the experiments.

Preparation of crude extracts : A portion weighing about 500 g each of the pulverized materials of SU, AU, RG, CR, CT and ZR was extracted by refluxing with 5 l of *n*-hexane three times, each time taking one hour. The residue obtained after filtration was then refluxed with ethanol by the same process. The extracts of the different solvents were each evaporated under reduced pressure to remove the solvents, and then liophylized for use in the tests.

Assays of color constituents : A suitable amount of each of the powdered crude extracts was accurately weighed and transferred to a flask to which 20 ml of 70 % methanol was added and the powder was extracted by ultrasonic vibration at room temperature for 15 minutes followed by shaking in a water bath at 40°C for 50 minutes. The extract solution from the flask was filtered through a microfilter (0.45 μm) into a 10-ml volumetric flask containing 1 ml of an internal standard solution (20 mg of acenaphthene dissolved 50 ml of 70 % methanol), filling up to volume with the extract. The solution in the flask was then shaken well to serve as a test solution. Each time 20 μl of the test solution was injected into the HPLC and the contents of the constituents were calculated according to the calibration equations worked out previously for the constituents. The HPLC operation conditions were as follows. Instrument : Waters high performance liquid chromatograph with Model 441 detector (λ = 254 nm) and Waters 510 pump. Precolumn : LiChroCART 25-4, LiChrospher 100RP-18 endcapped (5 μm), Merck. Column : μ-Bondapack C-18, 3.9 mm × 30 cm, particle size 10 μm, Waters. Mobile phase : acetonitrile : water : acetic acid (40 : 60 : 2). Flow rate : 1.0 ml/min. Sensitivity : 0.02 aufs. Chart Speed : 2.5 mm/min. The retention times and calibration equations (Y=ratio of peak heights, X=mg/ml) of the constituents were as follows. Curcumin=32 min., Y=4.9096X-0.0314 (r=0.9997) ; HCFM=30 min., Y=1.8433X-0.0261 (r=0.9998) ; DDCM=28 min., Y=1.8964X-0.1728 (r=0.9996).

Cholagogic activity test : By reference to the method reported by Yamahara, *et al.*,[9] the cholagogic effects of the crude extracts were carried out as follows. Normal Long-Evans rats, male, weighing about 200 g each, 7 rats per group, were selected and fasted for 6 hours prior to the operation. Under anesthesia with urethane, the rats were laparotomized and a polyethylene tubule was intubated into the common bile duct of each rat. After standing 1 hour for the rats to get stabilized, a definite dose of the aqueous suspension of the test sample was administered through the duodenum 30 minutes later, and at the ends of 30-minute intervals following the sample administration,

the bile output was measured. The measurement continued for 5 hours. Taking the bile output 30 minutes before sample administration as 100 %, the bile outputs at the ends of the individual 30-minute intervals were compared with it and the compared results were recorded to show the variation in bile secretion. In this test, the control group were given distilled water, and SDC was used as a reference compound.

Statistical analysis : The statistical means are presented with the associated standard errors in the form of "mean±S.E.". Student's *t*-test was utilized to determine the significance of differences between the control and the test (sample-administered) groups at the ends of the individual 30-minutes intervals.

Preliminary test : In order to find the most appropriate doses of the samples for intraduodenal (id.) administration, doses of 1,000, 2,000 and 4,000 mg/kg of the *n*-hexane extract of ZR (ZR-H) and of the ethanol extract of ZR (ZR - A) respectively were preliminarily tried for the cholagogic activity test.

Results

Table I shows the assay results of curcuminoid contents in the various turmeric and zedoary extracts. From the table, it is obvious that CR contains the highest amount of curcuminoids while RG contains the lowest. Generally, zedoaria contains less curcuminoids than curcuma rhizome or turmeric.

The results of the cholagogic activity test in the preliminary test are shown in Fig. 1 from which it is

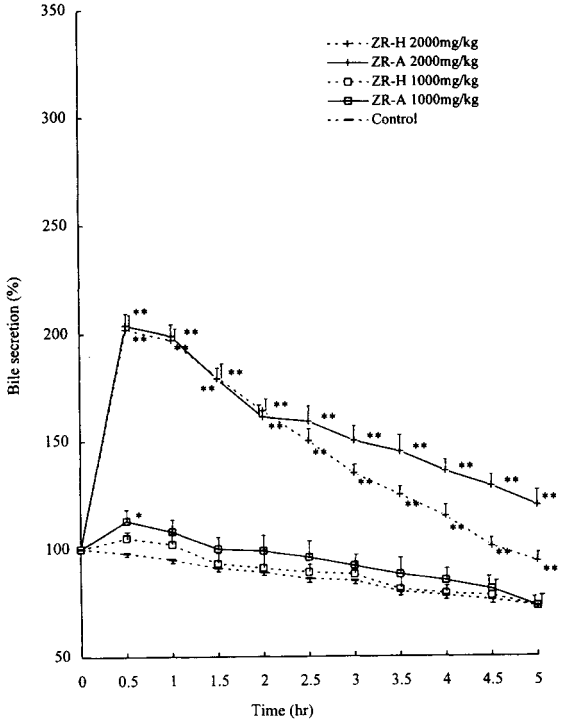

Fig. 1 Effects of Taiwanese zedoaria extracts on bile secretion in rats.
Each value is denoted in mean±S.E.(n=7). Significant differences from the control are expressed as $*p<0.05$ and $**p<0.001$. Samples were administered id. at 0 hr.

Table I Assays of curcuminoids in the various turmeric and zedoary extracts.

Sample	Curcumin (A) mg/g*	HCFM (B) mg/g*	DDCM (C) mg/g*	A+B+C (D) mg/g*	Yield of ext. (E) %	DxE mg/100 g**	Total %
SU-H	19.01	–	4.55	23.56	1.6	37.70	2.33
SU-A	266.22	82.23	83.51	431.96	5.3	2289.39	
AU-H	12.73	–	2.94	15.67	1.7	26.64	2.50
AU-A	262.86	87.14	92.11	442.11	5.6	2475.82	
RG-H	–	–	–	–	9.4	–	0.04
RG-A	7.64	3.40	1.86	12.9	2.9	37.41	
CR-H	15.39	–	–	15.39	4.3	66.18	8.85
CR-A	546.55	262.05	303.59	1112.19	7.9	8786.30	
CT-H	13.84	–	–	13.84	1.6	22.14	0.49
CT-A	106.39	40.53	49.82	196.74	2.4	472.18	
ZR-H	–	–	–	–	1.9	–	0.18
ZR-A	34.71	29.33	–	64.04	2.8	179.31	

"–" denotes undetectable. *denotes the amount in each extract.
** detotes the amount in each sample of crude herb material.

obvious that in the 1,000 mg/kg, id. groups, no significant effects on bile secretion from ZR-H and ZR-A could be observed. In the 4,000 mg/kg, id. groups, deaths were observed in part of the test animals during the 5-hour observation and therefore the data were discarded. From the results of the dose-response test shown above, the 2,000 mg/kg, id. dose seemed to be more suitable. Hence, this dose was used subsequently for the tests in this study.

The effects of the various turmeric and zedoary extracts on bile secretion are shown in Figs. 2 and 3. From these figures, we can see that in the control group the bile secretion decreased with time, whereas in the 2,000 mg/kg extract test groups, the bile secretion increased markedly. Bile outputs collected in the first 30-minute interval in the various test groups given the turmeric or zedoary extracts were increased to 2~2.5 times that before the sample administration. Although the bile secretion decreased with time, the cholagogic effect in these groups continued into the

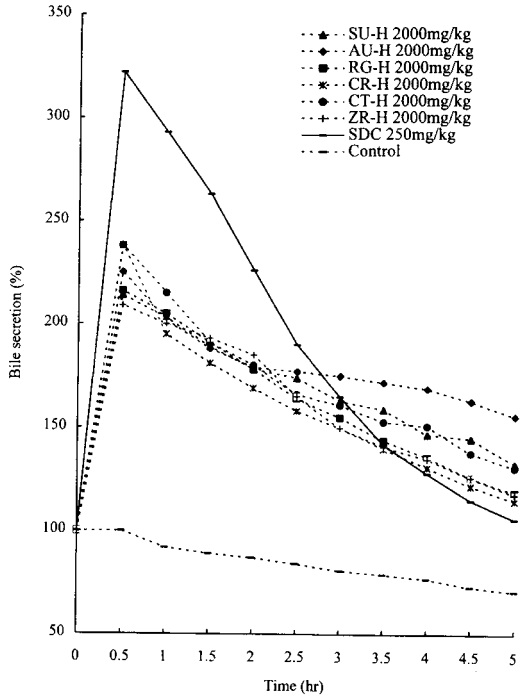

Fig. 2 Effects of *n*-hexane extracts of different turmeric and zedoary samples on bile secretion in rats.
Each value is denoted in the mean value (n=7). The±S. E. values are omitted for the sake of sparing space to make the figures clear. All the values obtained from the samples have statistically significant differences from the control at $p<0.001$. Samples were administered id. at 0 hr.

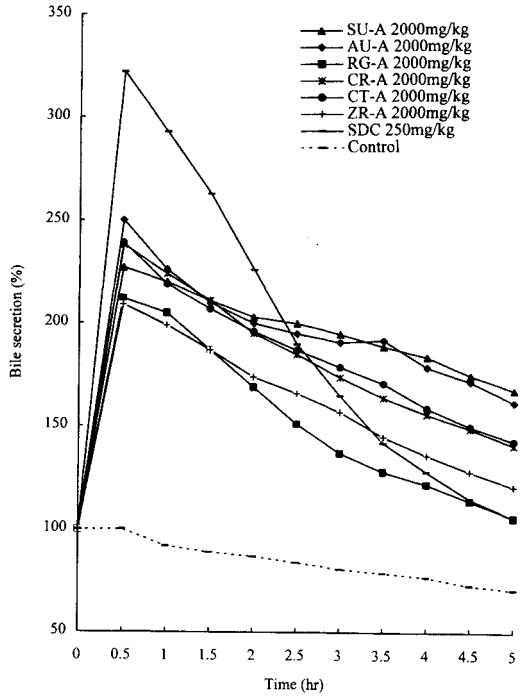

Fig. 3 Effects of ethanol extracts of different turmeric and zedoary samples on bile secretion in rats.
Each value is denoted in the mean value (n=7). The±S. E. values are omitted for the sake of sparing space to make the figures clear. All the values obtained from the samples have statistically significant differences from the control at $p<0.001$. Samples were administered id. at 0 hr.

fifth hour at the end of which the output was still maintained at 106-168 %. On the other hand, in the group given SDC (dosed at 250 mg/kg), the bile secretion in the first 30-minute interval was elevated up to 322 % (mean value) though, the secretion dropped rapidly with time and by the end of 2.5 hours following the administration of SDC, the output dropped down to 190 % and by the end of the fifth hour, the output downed to only 106 %. Hence the maintenance of drug efficacy of SDC did not seem so good as turmeric or zedoary extracts.

Among the 12 test groups, the ethanol extracts of SU (SU-A), AU (AU-A), CT (CT-A) and CR (CR-A) showed superior cholagogic effects in that their bile outputs by the end of 2.5 hours were 2 times or nearly 2 times higher, and by the end of the fifth hour, the bile outputs were still maintained 1.5 times more or nearly 1.5 times as high as before the sample administration. In these 4 extracts, the curcuminoids contents were higher than in the other extracts as

shown in Table I. On the other hand, the *n*-hexane extracts of RG (RG-H) and ZR-H did not contain curcuminoids though, nevertheless they also exhibited a certain extent of cholagogic effect.

In another experiment, when each extract in the dose of 2,000 mg/kg was orally administered to the rats (three rats per group) individually, only very weak to moderate responses in terms of behavior depression and muscle relaxation were observed during the first 3 hours in those groups of rats which had been administered with *n*-hexane extracts of AU (AU-H) and CR (CR-H), RG-H and ZR-H, and no death was observed during the 72-hour observation period.

Discussion

Turmeric and zedoaria have been used as an aromatic stomachic or cholagogue for a long time. The results of the present study have shown that the various extracts of turmeric and zedoaria can certainly promote the bile secretion which is essential for digestive absorption and intestinal alteration, and therefore reassure their use as an effective stomachic or cholagogue. As the ethanol extracts and *n*-hexane extracts of the various turmeric and zedoary samples were administered to rats in a dose of 2,000 mg/kg. id., they produced different degrees of cholagogic effect, but the patterns of cholagogic effects among the samples were similar in that in the first 30-minute interval following sample administration, the bile output markedly reached the climax and then declined with time, the effect lasting for about 5 hours. Usually curcuma rhizome or turmeric have more marked cholagogic effect and have higher contents of curcuminoids than zedoaria. In particular, the ethanolic extracts of the former have more marked cholagogic effect and have higher curcuminoid contents than its *n*-hexane extracts. For example, AU-A, SU-A, CT-A and CR-A possess stronger and sustaining cholagogic effects and their curcuminoids contents are also higher, which may be indication that the cholagogic effect with these herbs might be curcumimoids related. However, since the RG-H and ZR-H extracts which do not contain curcuminoids also can exhibit a certain extent of cholagogic effect, we may postulate that there could be some other constituents contained in these herbs such as the essential oils that may promote bile secretion. The results of the study also have shown that among the various samples of turmeric and zedoary, the Japanese "ukon" or SU and AU demonstrated the most marked cholagogic effect. As for the pure compounds of curcumin, HCFM and DDCM, the cholagogic activity tests for these compounds were not carried out, owing to insufficient amounts and poor stability of the compounds. While the cholagogic mechanism with turmeric and zedoary is still under investigation, further details in this aspect will be reported later on.

Conclusion

The cholagogic effects of some commercial articles of turmeric and zedoary were studied. The results have shown that all the *n*-hexane and ethanol extracts of these articles possess marked cholagogic effects significantly different from that of the control ($p<0.001$) when administered in a dose of 2,000 mg/kg, id. in the rat, especially the AU-A, SU-A, CT-A, and CR-A extracts which have stronger and more lasting cholagogic effects and contain higher contents of curcuminoids than the other extracts.

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We are deeply indebted to Nihon Ukon Sangyo Co., Ltd. for their generous donation of the commercial articles of SU, AU, and RG.

和文抄録

市販春ウコン（SU），秋ウコン（AU），琉球ガジュツ（RG）及び台湾市販の姜黄（CR），郁金（CT），莪朮（ZR）の胆汁分泌作用に関する検討を行った。これらウコン，ガジュツのヘキサン及びエタノール抽出乾燥エキスを試料とし，試料を各2,000 mg/kg, id. 投与した各群ラットの試料投与後30分間の胆汁分泌量は投与前30分間の分泌量の約2～2.5倍に増加がみられ，かつ持続的な胆汁分泌促進作用がみられた。これら試料のうちSU, AU, CT及びCRのエタノール抽出乾燥エキス（SU-A, AU-A, CT-A及びCR-A）4試料を投与した各群には特に胆汁分泌促進作用が顕著にみられ，かつ2.5時間後まで約2倍又は2倍近く，5時間後まで1.5倍以上又は1.5倍近

くに長時間の促進作用がみられた。

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