Analysis on the *Alpinia katsumadai* components of *Zingiberaceae* plants and their functions on myeloma resistance

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Abstract: Generally speaking, zingiberaceae plants with sweet fragrance are commonly seen as perennial herbs that contains numerous well-known crude drugs and fragrant plants like *Amomum villosum, Amomum tsao-ko*, Ginger, *Alpinia katsumadai* and *Radix curcumae*, which are widely used in daily life. This paper analyzed chemical components of *Alpinia katsumadai* of zingiberaceae and applied several laminar analysis to further develop its active ingredients, aiming to make sure its function on tumor assistance. Actually, cardamomin contained in *Alpinia katsumadai* has been recorded to act notably in myeloma resistance, which was verified by cholecystokinin-octopeptide (CCK-8) in this paper. Cardamom in is proved to have multiple anti-myeloma effects, including myeloma cell activity and proliferation control, cell cycle retardant and apoptosis induction, which indicates its value in the field of medical pharmacy.

Keywords: *Zingiberaceae* plant; cardamom in; multiple myeloma (MM) cell; cell cycle.

INTRODUCTION

As modern medicine develops, chemicals with great side-effect and drug-resistance can not catch up with the increasing demand of health preserving. On the other hand, active compound and lead compound of natural compounds are found with novel structure, high curative effect and little side-effect, so they have been taken as major models for new drug design (Isabelle *et al*., 2010). Alpinia plants are rich in diarylheptanoid, flavonoid and oil, among which former two have wide pharmacological activity. To be specific, diarylheptanoid acts actively in oxygen resistance and plays functions on tumor, inflammation and emetics resistance; and flavonoid has an obvious impact on inflammation and tumor resistance. That is why diarylheptanoids and flavonoids attract lots of attention from phytochemistry and medicine day by day. He Jiake *et al*. (Jiake *et al.*, 2010) stated in details about flavonoid (natural plant) components, and generalized metabolic features of flavonoid medicine, which was significant in improving safety of clinical pharmacy and promoting development and clinical application of flavonoid medicines. Qi Shufen and Yao Qingqiang (Shufen and Qingqiang, 2009) reviewed research progress on chemical component and pharmacological activity of alpinia plants that were developed and applied in a better way. Besides, Qin Huazhen and Wang Xiaoqian (Huazhen *et al.*, 2010) looked for relevant papers on pharmacological effect of alpinia plants and summarized research progresses on the effects, offering proof for further study and development of Chinese medicines made of Alpinia plants.

Based on selection and determination, the paper figured out obvious anti-multiple myeloma (MM) effect of cardamom in, according to which, further studies adopting multiparty determination were carried out to prove influence of cardamom in on controlling MM cell activity and proliferation, inducing apoptosis and blocking cell cycle, which firmly built theoretic basis for cardamomin's future research and development.

MATERIALS AND METHODS

Experimental materials

(1) Cell strain: human MM cell strains including RPMI8226 and U266 were kept in the laboratory.
(2) Plant specimen: dry seeds of *Alpinia katsumadai* of alpinia plant of zingiberaceae.
(3) Experimental reagent: standard cardamomin identified by National Institutes for Drug and Biological Product Control.

Methods

Extract and separation of chemical components contained in Alpinia katsumadai

A total of 4kg dry Alpinia katsumadai fruits was smashed into coarse powders and mixed with 80% alcohol that was 5 times the volume of Alpinia katsumadai, followed by heating to maintain extract solution. The extract was repeated for 3 times and each time for 3 hours. Then extract solutions were mixed to obtain 476.0g extract with decompression and concentration, which was extracted by aether petrolei, acetate ester and n-butyl alcohol. When this was done, acetic ether maintained was made to separate principle chemical components by various chemical methods. All steps are shown in fig. 1.

Human MM cell culture

Human MM cell strains, namely RPMI 8226, ARH-77 and U266 were cultured according to regular culture method of suspension cells, meaning that cell strains were cultured on RPMI 1640 complete medium with 10% FBS, 100 U/ml penicillin and 100mg/L streptomycin under the

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condition of 5% CO_2 and saturated humidity under 37°C. Every one-day or two days, cell RPMI 8226 and ARH-77 went down to posterity, and U266 went down to posterity once each three to five days. Besides, all cell strain solutions were changed in half 24 hours before the experiment, followed by trypan blue dyeing identifying 98% cell activity.

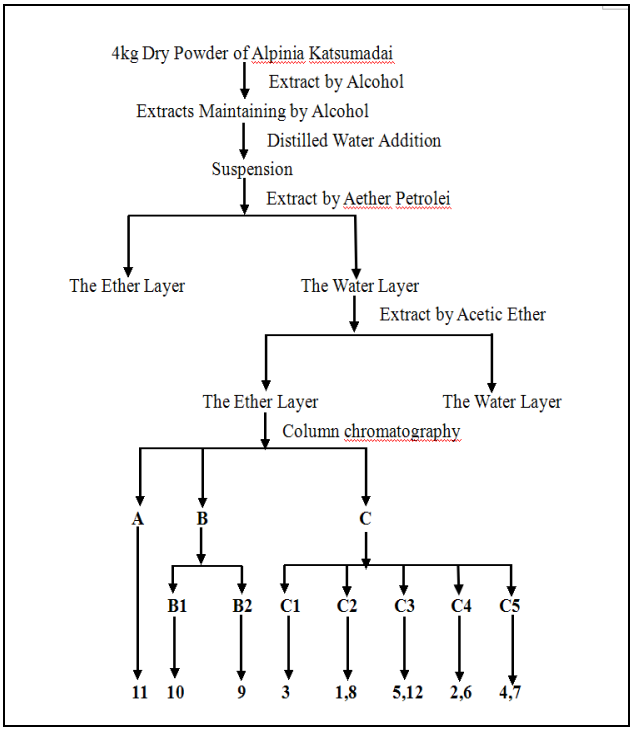

Fig. 1: Extract and separation of chemical components in *Alpinia katsumadai.*

Research on anti-myeloma mechanism

Having taken human MM cells as major cell strains in the experiment, medical intervention of cardamom in reagent was complemented to observe formal changes of MM cells. In addition, cell activity was determined by CCK-8, cell proliferation by EdU, cell cycle by propidium (PI) single dyeing and cell apoptosis by Annexin V FITC/PI double dyeing.

STATISTICAL ANALYSIS

SPSS 19.0 software was performed in statistical analysis, and regular test for normality and homogeneity of variance was carried out. Measurement data was presented as mean ± S.D., while t test was adopted in the comparison of single variance that was verified by non-parameter test once it was not equal. If $P<0.05$, differences were considered as statistically significant.

RESULTS

Chemical components analysis of Alpinia katsumadai

After purified and separated by several means of laminar analysis, acetic ether was supposed to have chemical component analysis, leading to 12 monomeric compounds, (3S, 5S)-trans-3, 5-dihydroxy-l, 7-diPhenyl-hePt-ene (1), (3R, 55)-trans-3, 5-dihydroxy-l, 7-diphenyl - hevt-l-ene(2), 5-hydroxy-l, 7-diphenyl-hepta-6-en-3-one(3), cardamom in (4), alpinetin (5), pinocembrin (6), pinostrobin (7), naringenin (8), (+)-catechin (9), chrysin (10), rutin (11), 2,4-dihydroxy-6-phenemyl-methyl benzenecarboxylate (12), among which the last five compounds were firstly discovered in Alpinia katsumadai. Moreover, cardamom in was stated in literature (S. Vincent, 2010; Shangqin *et al*., 2010) to have sound anti-tumor effect, which would be a study focus in the future.

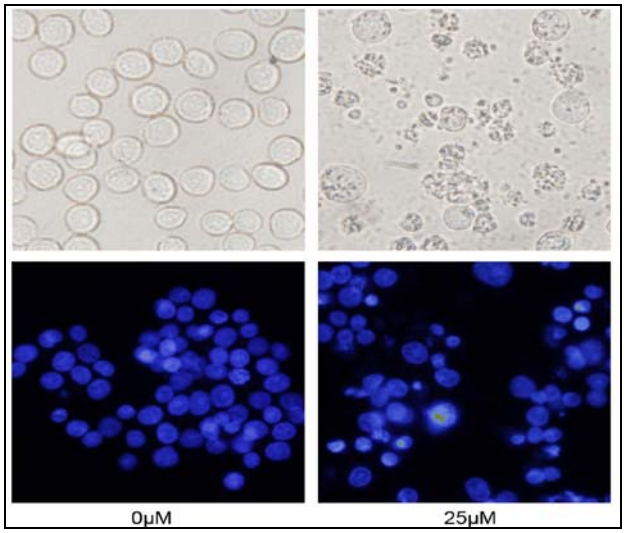

Fig. 2: Influence of cardamom in on MM cell morphology

Influence of cardamom in on MM cell morphology

RPMI 8226 cell strains were processed by cardamom in with 50μM concentration, which after 24 hours was made to observe MM cells' formal changes. Under fluorescence microscope, different morphological lesions occurred to RPMI8226 cells, such as lessened volume, increased cytoplasm, broken kernel, narrowed nucleoplasm, and even apoptosis of globule. Please refer to fig. 2.

Influence of cardamom in on human MM cell activity

Cell strains, referring to RPMI 8226, ARH-77 and U266 were intervened by crdamomin with different concentrations (0, 10, 20, 30, 40, 50, 60, 70, 80, and 100μM) for 48 hours, and their activity was determined by CCK-8. Compared to MM cells without cardamom in reagent, cells with intervention had remarkably reduced activity, meaning that cardamom in controlled MM cell activity in a stronger way when concentration increased, which obviously depended on time passage. In details, 10μM cardamom in led to 47.30±14.11% survival rate in RPMI 8226 cells, 48.74±0.76% in ARH-77 and 59.05±1.77% in U 266; while with 100 μM concentration, the rate was respectively 14.00±3.88%, 3.96±0.23% and 1.40±0.75%, which is presented in fig. 3.

Table 1: Influence of cardamom in on MM cell cycle

Cardamomin (μM)	RPMI 8226			U266			ARH-773		
	G1	G2/M	S	G1	G2/M	S	G1	G2/M	S
0	31.34%	14.62%	54.05%	48.52%	13.39%	38.09%	43.59%	11.13%	44.92%
10	31.25%	51.53%	17.21%	44.94%	20.86%	34.19%	31.84%	19.93%	48.24%
20	4.12%	81.89%	14.00%	14.29%	50.00%	35.71%	32.23%	39.26%	28.24%

Influence of cardamom in on MM cell (RPMI 8226) proliferation

The paper researched on RPMI8226 to analyze how cardamom in affecting MM cell proliferation. To be specific, RP MI8226 was processed by 50μM cardamom in for 24 hours and observed by EdU. As shown in fig. 4, if RPMI8226 was intervened by cardamom in, positive cells, namely newly reproduced cells, greatly decreased, which indicates reduced activity of cell proliferation.

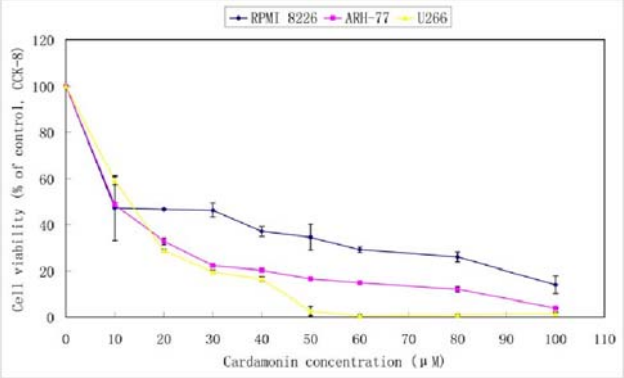

Fig. 3: Influence of cardamom in with different concentrations on MM cell activity

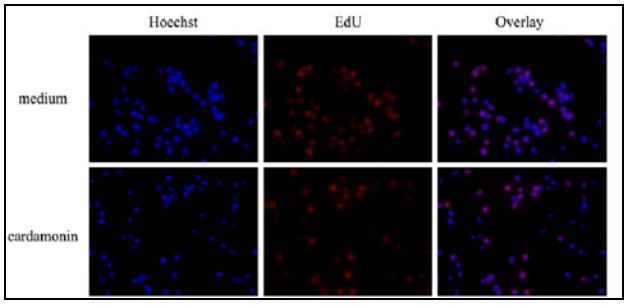

Fig. 4: Influence of Cardamom in on RPMI 8226 Cell Proliferation

Influence of cardamom in on MM cell cycle

With 24-hour cardamom in intervention (0, 10 and 20 μM), MM cell cycle changes were determined by flow cytometry with PI single dyeing, whose results are presented in table 1. As concentration rose up, MM cell cycle distributions varied accordingly, performing as cell increase in stage G2/M and cell decrease in stage S.

Influence of cardamom in on human MM cell apoptosis

After cardamom in with 0, 20, 50 and 100μM concentration intervened MM cells for 24 hours, Annexin V FITC/PI double dyeing was applied to determine apoptosis rate, leading to increase of positive cell (apoptotic cells in early and later stage) rate with increasing concentration. In details, apoptotic cell rate in RPMI 8226 cells went up from 5.3% (0μM intervention) to 91.14% (100 μM) with increase of 18 times; U266 cells witnessed 8 times rise from 9.5% to 83.6%, and ARH-77 cells were found with 4 times increase from 25.6% to 95.6%.

DISCUSSION

Natural Chinese medicinal herbs possess plenty of components with anti-tumor activity, from which highly effective anti-tumor components can be extracted to develop new anti-tumor drugs. This is proved to be practicable (Quanxing and Xiaokang, 2011). In the past times, Chinese scholars successfully selected various drugs for neoplastic hematologic disorder treatment, such as arsenious acid, retinoic acid and indirubin. Widely applied in clinic, those natural drugs display notable curative effects, which is a hope for patients suffering from cancers of the blood.

Cardamom in with biological activities is from chalcone of Chinese medicinal herbs, which functions on spleen strengthening and dampness dry, and stomach warming and vomiting control. Besides, it does good for relieving cold resistance, abdominal fullness and distention, crymodynia, belching and retching counterflow, and poor appetite. However, its effect on tumor resistance is not reported and not widely known by the public (Vivek R *et al*., 2011; Angelo and Domenico, 2011). So this paper focused on cardamomin's anti-myeloma effect, expecting to build firm foundation of cardamomin's clinical application.

Drugs control MM cell activity from two aspects; one is cell proliferation and cell cycle control, and the other refers to cell apoptosis or necrosis induction. As in the experiment, EdU was firstly adopted to observe how cardamomin functioning on MM cell proliferation, which could be identified by rate change of positive cells before and after drug intervention. As a result, cell proliferation was significantly controlled by cardamomin.

A complete cell cycle starts from the completeness of last fission and ends with the completeness of next fission, which can be divided into five stages, G0 (quiescent stage), G1 (early DNA synthesis), S (synthetic phase), G2 (postsynthetic stage), and M (mitosis). In addition, G1/S

and G2/M are two major restriction points. In this paper, PI single dyeing was the main method determining influence of cardamom in on MM cell cycle (Hae-Yun *et al.*, 2011). It was noticed that with little dosage, cardamom in blocked MM cell in stage G2/M, which was regulated by dosage dependence.

Apart from above, how cardamom in affecting MM cell spoptosis was detected by Annexin V/PI double dyeing, suggesting that cardamom in mainly induced early cell apoptosis depending on dosage with extremely low necrosis rate. But compared to cardamom in concentration blocking cell cycle, concentration inducing MM cell apoptosis was much higher, which showed that small dose of cardamom in reagent blocked MM cells to slow down cell proliferation, and large dose directly caused cell apoptosis.

CONCLUSION

In conclusion, by principal chemical component analysis of Alpinia katsumadai, this paper does study on cardamom in, a anti-MM drug with stronger activity. With several effective determination methods like CCK-8, EdU and PI single dyeing, cardamomin is found to remarkably control MM cell activity and proliferation, block cell cycle in stage G2/M, and induce early apoptosis. What mentioned above provides clinical application of natural herbs resisting cancer with powerful facts and theoretic basis.

REFERENCES

Angelo V and Domenico R (2011). Angiogenesis and vasculogenesis in multiple myeloma: Role of inflammatory cells. *Recent Results Cancer Res*., **183**: 87-95.

Hae-Yun C, Eun-Kyoung C, Soo-Woon L, Ki-Hyung K, Sang-Jun P, Chae-Kwan L and Soo-Woong L (2011). All-trans retinoic acid induces TLR-5 expression and cell differentiation and promotes flagellin-mediated cell functions in human THP-1 cells. *Immunol. Lett*., **136**: 97-107.

Huazhen Q, Xiaoqian W, Junhui L and Lei L (2010). Research Progress on Pharmacological Action of Chinese Medicines Belonging to Alpinia. *Guangxi. J. of Tradit. Chin. Medi*., **33**(5): 1-3.

Isabelle A, Rachel B and Francoise D (2010). New treatments for myeloma. *Joint Bone Spine*, **77**: 20-6.

Jiake H, Yang Y, Xijing C, Wei S, Fang F, Ning L and Jianheng Z (2010). Research Progress on Drug Metabolism of Flavanoids. *China. J. of Chin. Materia. Medi*., **35**(21): 2789-2794.

Quanxing W and Xiaokang L (2011). Immunosuppressive and anti-inflammatory activities of sinomenine. *Int. Immunopharmacol*., **11**: 373-376.

Shufen Q and Qingqiang Y (2009). Review on the Chemical Constituents and Pharmacological Activities of Alpinia L. *J. of Pharm. Res*., **28**(5): 284-287.

S Vincent R (2010). Multiple myeloma: 2011 update on diagnosis, risk-stratification, and management. *Am. J. Hematol.*, **86**: 57-65.

Shangqin L, Zi M, Huili C, Qian L, Wenyu R and Michio K (2010). Inhibitory effect of baicalein on IL-6-mediated signaling cascades in human myeloma cells. *Eur. J. Haematol*., **84**: 137-144.

Vivek R Y, Sahdeo P, Bokyung S and Bharat B A (2011). The role of chalcones in suppression of NF-κB-mediated inflammation and cancer. *Int. Immunopharmacol*., **11**: 295-309.