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Cytotoxic activity of Hommali 105 rice bran extract against human cancer cells

Sakuntala Uttama[1*], Arunporn Ittharat[2]**

[1] Student of Doctor of Philosophy, Faculty of Medicine, Thammasat University.
[2] Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University.
*Presenting Author, ** Corresponding author

Abstract

The objectives of this research are the investigation for the cytotoxic activity against cancer cells of *Hommali 105* rice bran (*Oryza sativa*) extracts by seven difference extraction methods. The methods of extraction were maceration by 95% ethanol, supercritical fluid extraction, boiling in water, freeze dry expression method and Soxhlet extraction by using different solvent such as hexane, chloroform and methanol. The highest percentage of yield derived from the water extract was 21.7. Cytotoxic activities screening by the SRB assay were carried out against four human cancer cell lines: lung (CORL23), cervical (Hela), prostate (PC3) and breast (MCF-7) and normal human cells line (MRC5). The results found that the chloroform extract showed the highest inhibition against prostate cancer cells, followed by cervical and breast cancer cells (46.9%, 29.6% and 21.58%, respectively), but it had no cytotxic activities against both lung cancer and normal cells . This extract is also selective cytotoxic activities against only human cancer cells depend on hormone such as prostate (PC3) cervical (Hela) and breast (MCF-7) cells.

Keywords: Hommali 105 rice bran , *Oryza sativa* , the extraction, cytotoxicity, SRB

Introduction

Cancer is the first leading cause of death of Thai people nowadays. It is suffer for treatment cancer patients by chemotherapy which can kill both cancer and normal cells. Thus the research try to find out drug which can kill cancer cells but not show cytotocic activity with normal cells. Plants were used to treat cancer for long time ago and it was isolated acive cytotoxic compounds and derived them to against cancer cell such as pacitaxel from Pacific Yew tree. Two types of hydroxy acids were isolated from water extract from rice bran [(10*E*, 12*Z*)-9-hydroxy-10,12-octadecadienoic acid [A] and (9*Z*, 11*E*)-13-hydroxy-9,11-octadecadienoic acid [B]], exhibited cytotoxic against P388 mouse leukemia cells The cytotoxicity of acid A was stronger than that of acid B . [(1)]. From this research make to continuous research about rice bran extract to use to treat cancer cells because rice bran is popular use for health food in Thailand and rice is a main food of Thai people long time ago. Rice bran is prepared as several product for health tonic. Its powder has previose reports as high nutritive food and used for reducing blood cholesterol, antioxidative properties, decreasing the incidence of atherosclerosis disease and having laxative effect. The components of rice bran composed with sterol , gamma-oryzanol, tocopherols, tocotrienols and phenolic compound [(2)]. In Thailand the best rice and popular is Hommali rice and the type is 105. Thus, the objective of investigation is to test cytotoxic activity against four types of human cancer cells of rice bran extract which there is no previous report for cytotoxic study against cancer cell from rice bran extract. Type of the rice bran is homali 105 as the most popular use in Thailand.

Methods

Plant materials and extraction methods

Hommali 105 rice bran used in this study was collected from Surin province, Thailand. Five exraction methods are maceration by ethanol 95%, supercritical fluid extraction, boiling in water extraction and dry by frreeze dryer , expressed extraction method and soxhlet extraction method by using solvent hexane, chloroform and methanol. We got seven extract for cytotoxic testing The percentage of yield showed in table 1

In vitro cytotoxicity testing and SRB assay

The cytotoxicity assay was carried out using sulphorhodamine B (SRB) assay (2). The target cell lines were four type of human cancer cells i.e. lung (COR-L23), prostate (PC3), breast (MCF-7), and cervical (Hela) cancer cell lines and one type of human normal lung cell (MRC5). The 100μg/ml of seven different extracts were tested against all cell lines by SRB assay. The culturing of the cancer cells was as described by Keawpradub *et al*. (1999) [3] and Itharat *et al*. (2004) [4] while the MRC-5 cells were cultured as described by Itharat *et al*. (2004) [4]. The active plants extracts were diluted and tested for calculating IC_{50}.

The monolayered culture of each cell line in a 96-well microtiter plate was treated with each plant extracts for 6 replications. The plates were incubated for an exposure time of 72 hours, then the medium was removed and washed. The plates were incubated for a recovery period of 6 days. The percentage of toxicity was measured colorimetrically using SRB assay and IC_{50} values was calculated by means of Prism program.

Results and discussions

Boiling in water method and dry by freeze dired showed the highest of percentage of yield as 21.73 % and the second is from Supercritical fluid extraction is 8.57%.

Table 1 percentage of yield of rice bran from Hommali 105 extract on different extraction methods

	Code of extract	Method of extraction	% Yield
1	ME	Maceration with ethanol 95%	3.09
2	SUP	Supercritical extraction	8.57
3	BOIL	Boiling in water , filtrate and dry by freeze dryer	21.73
4	EX	Expression	7.00
5	SOXME	Soxhlet extraction by hexane	6.58
6	SOXCH	Soxhlet extraction by chloroform	1.09
7	SOXHE	Soxhlet extraction by methanol	6.58

The results of cytotoxic activity screening showed in table 2 . It was found that soxhlet extraction by chloroform extract showed the highest percentage of inhibition against prostate cancer cells , followed by cervical and breast cancer cells (46.9, 29.6 and 21.58 respectively) but it had no cytotxic activity against both lung cancer and normal cells . This extraction method also is selective cytotoxic activity against only human cancer cells depend on hormone such as prostate (PC3) cervical (hela) and breast (MCF-7) cells

Table 2 Percentage of cytotoxic activity (% Toxicity ± SD)or inhibition of growth all types of cell lines

Extraction methods	CORL-23	Hela	PC3	MCF7	MRC5
MEt	12.05 ± 0.23	-0.21 ± 0.02	-11.95 ± 0.04	**20.53± 0.03**	-19.84±0.03
SUP	**31.14 ± 0.46**	5.54 ± 0.08	2.33 ± 0.04	15.79±0.01	-18.65±0.02
BOIL	**13.04 ± 0.01**	-7.71 ± 0.07	-2.69 ± 0.02	8.70±0.03	-6.55±0.03
EX	8.84 ± 0.20	-2.77 ± 0.07	5.83 ± 0.04	6.84±0.02	-26.98±0.03
SOXHE	0.31 ± 0.04	3.80 ± 0.05	9.62 ± 0.02	15.26±0.02	-28.17±0.02
SOXCH	-10.22 ± 0.08	**29.57 ± 0.05**	**46.94 ± 0.04**	**21.58±0.01**	-8.73±0.04
SOXME	-0.78± 0.09	-2.77 ± 0.05	-4.96 ± 0.05	10.00±0.00	-7.94±0.05

The supercritical fluid extract of rice bran showed also selective cytotoxic against lung and breast cancer cell , less active for cervical and prostate (31.1, 15.8, 5.5 and 2.3% respectively) but this extraction method made normal cells proliferate growth. It is good thing to used this extract for promoting to used in lung cancer patients because it can kill lung cancer but not kill lung normal cells. In addition to macearation method showed also selective cytotoxic against breast and lung cancer cells but it promote growing or proliferation prostate and cervical cell growth.

The rice bran oil from expression showed proliferation normal cell but less than soxhlet extraction by hexane. Characteristic of two extract are oil it showed cytotoxic activity against cancer cell but proliferate normal cells so they should be promoting using rice bran oil in cancer patients. Free fatty acid in these oil should be continuous studied because the previous report (1) found that free fatty acid exhibited cytotoxic against P388 mouse leukemia cells

However, all of method showed less cytotoxic against all type of cancer cell because IC50 value are more than 100 μg/ml The benefit of this study showed that the extraction method make cytotocic against cancer cell difference. Thus, the methods for preparing rice bran to be product for cancer will be chosen appropriately with type of cancer cells such as the soxhlet extraction by chloroform appropriate with prostate and cervical cancer cells , supercritical fluid extraction is the best for lung cancer cells. Rice bran oil from soxlhlet extraction by hexane and expression are good for promote lung normal cell growth

Conclusion

The highest of percentage of yield is water extract by boiling method .The soxhlet extraction by chloroform extract showed the highest percentage of inhibition against prostate cancer cells , followed by cervical and breast cancer cells but it had no cytotxic activity against both lung cancer and normal cells . This extraction method also is selective cytotoxic activity against only human cancer cells depend on hormone such as prostate (PC3) cervical (hela) and breast (MCF-7) cells

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References

1. Hayashi, Y., Nishikawa, Y., Mori, H., Tamura, H., Matsushita, Y. and Matsu, T. Antitumor Activity of (1OE, 12Z)-9-Hydroxy- 10, 12-Octadecadienoic Acid from Rice Bran J. Fermentation and Bioengineering. 1998; 86 (2): 149-53.
2. Chotimakorn, C., Benjakul, S. and Silalai, N. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chemistry, 2008; 111: 636-41.
3 Keawpradub, N., Amooquaye, E.E., Burke, P.J. and Houghton, P.J. Cytotoxic activity of indole alkaloids from *Alstonia macrophylla*. Planta Medica 1999; 65: 311–15.
4 Itharat, A. Houghton, P.J., Eno-Amooquaye, E., Burke, P. J., Sampson, J.H. and Raman, A. *In vitro* cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J. of Ethnopharmacology 2004; 90:33-8**.**