Introduction

Cervical cancer is the second most incidence of cancer in women next to breast cancer. From the report of National Cancer Institute or NCI of Thailand, there are 6,000 cases of new cancer patients with the average dead of 7. The incidences of cervical cancer were found in women at the age of 35-50 years old. At present, there are many anti-cancer drugs from natural sources, for examples, Paclitaxel which is used to treat breast cancer and has been developed from a plant called, Pacific Yew and vincristine which is used to treat leukemia which has been developed from a plant called, Periwinkle. For cancer treatment, the research and development of a new anticancer drug from medicinal plants appeared in the medicinal plant recipes will have high chance to get a new high efficient anti-cancer drug. This may be due to the traditional use which has been proved to be effective and be recorded and used until now.

Thailand and other ASEAN countries have the long history of folklore wisdoms in using medicinal plants for treating diseases. Many are used both as single plants or mixtures in the recipes. These knowledges have been recorded in various documents such as palm leaves, mulberry pulp paper and *Streblus asper* paper. Prof. Dr. Jiradej Manosroi and his team have collected the Thai medicinal plant recipes from all over Thailand, and established the "Manosroi III" database (Figure 1) since 1993. Up till now, the recipes which has been translated, checked for correctness and recorded in the database are about 85,000 recipes. When completed, the total recipes are estimated to contain in the database of about 200,000 recipes. By using the keywords to search for the targeted recipes, there are 687 anti-cancer recipes found in the database.

Figure 1 The Thai medicinal plant recipe database "Manosroi III"

From the anti-cancer research project supported by the Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health in Thailand of 3 phases during 2009-2014, the 221 anti-cancer recipes have been selected from the "Manosroi III" database using the keywords of cancer and tumor. These recipes have been pre-clinically investigated, including the *in vitro* anti-cancer activities in cancer cell lines and xenograft nude mice. The safety of the recipes was also tested in animal models. It has been found that the selected recipe N040 does not only show the anti-cancer activity of cervical cancer both in *in vitro* and animal model, but also has no toxicity in animals.

The N040 Thai medicinal plant recipe from the Manosroi III database is originally from the Thai Lanna region. It is composed of medicinal plants from the northern part of Thailand (Table 1).

Table 1 Descriptions of the anti-cancer Thai medicinal plant recipe N040

Recipe no.	Source	Preparation	Example of plants compositions in the recipe
N040	Chiang Rai.006-093 /87 156 08 052-052 /0103	Take plants compositions in the recipe, knead together and eat, can cure cancer	*Urceola minutiflora* *Sida rhombifolia* *Plyalthia debilis* *Nymphoides indicum* *Psophocarpus tetragonolobus*

Extraction of the Thai medicinal plant recipe N040

For extract preparation, all plant compositions in N040 recipe were ground, mixed, boiled, filtered to remove the residues, evaporated to concentrate and dried. The dried extract of N040 recipe was in light brown color (Figure 2), slight pungent smell and has the yields of 10%. For the phytochemistry test, the N040 recipe extract showed positive test of flavonoids, tannins and alkaloids.

Figure 2 The appearances of the Thai medicinal plant recipe N040 extract

Anti-cervical cancer activity of recipe N040 extract

1. **Anti-proliferative activity on human cervical adenocarcinoma (HeLa) by the sulforhodamine B (SRB) assay**

The N040 recipe extract showed the anti-cancer activity on HeLa cell line with the IC_{50} value of 0.11 µg/ml which was more potent than the gold standard anti-cancer drug, cisplatin of 31.09 times **(Table 2)**.

Table 2 Anti-proliferative activity on HeLa (IC_{50} (µg/ml)) of recipe N040 extract from SRB assay

Recipe no.	IC_{50} (µg/ml)	Folds of the standards			
		Doxorubicin	Vincristine	Cisplatin	5-FU
N040	0.11±0.03	24.91	0.18	31.09	10.64

2. **Apoptosis induction on HeLa by acridine orange (AO) and ethidium bromide (EB) staining**

The N040 recipe extract gave the apoptosis activity of HeLa cancer cell line **(Table 3)**.

Table 3 Percentages of apoptotic cells of the HeLa cell line treated with the recipe N040 extract by AO/EB staining

Recipe no.	% apoptotic cells	Folds of the standards			
		Doxorubicin	Vincristine	Cisplatin	5-FU
N040	1.35±1.18	0.18	0.45	1.50	0.22

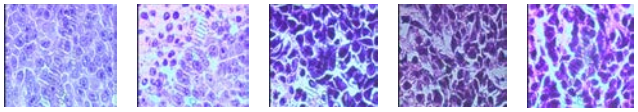
3. ***In vivo* anti-cervical cancer activity by the HeLa xenograft nude mice model**

The extract was tested for anti-cancer activity in nude mice which are mice without immunity and xenografted with the HeLa cancer cell line. After fed with the extract for 14 days, the N040 extract at all doses can inhibit the tumor on the nude mice, at the low dose, medium dose, high dose, in comparing with the control group which was not fed with the extract and the positive control fed with the gold standard anti-cancer drug, cisplatin **(Figure 3)**. The percentages of the tumor weight inhibition of the xenograft nude mice fed with the medium doses of the N040 extract gave the highest value of 57.23% which was about 65% of cisplatin **(Table 4)**. For histopathology examination of the tumor in the treated xenograft nude mice at high and medium doses of the N040 extract, the atrophy of the cancer cells with the fragmented nucleus **(Figure 4)** indicating of apoptosis was observed.

Figure 3 Sizes of tumors in the nude mice after the treatment for 14 days

Table 4 Effects of the recipe N040 extracts on HeLa cell xenograft nude mice model after the treatment for 14 days

Group	Tumor weight (g)	% inhibition of tumor weight	Folds of cisplatin
Control	3.78±0.24	0.00	0.00
N040 low dose	2.52±0.39	33.26	0.38
N040 medium dose	1.62±0.24	57.23	0.65
N040 high dose	1.94±0.28	48.67	0.55
Cisplatin	1.55±0.97	87.83	1.00



(A) (B) (C) (D) (E)

Figure 4 Histopathology of tumor mass of HeLa cell xenograft nude mice model after the treatment for 14 days. (A-C) N040 at low dose, medium dose and high dose (D) control (sterile normal saline, p.o.) and (E) cisplatin (5 mg/kg body weight per week, i.p.).

Safety of N040

The N040 recipe extract was tested for safety in Wistar rats, by the sub-chronic assay. The rats in both sexes were fed daily for 3 months with the extract at 1 g/body weight. The hematology and biochemistry assay of the blood from the rats after the experiment were normal. The histopathology examination of all organs including brain, heart, spleen, small intestine, colon and reproductive organs were also normal **(Figure 5)**.

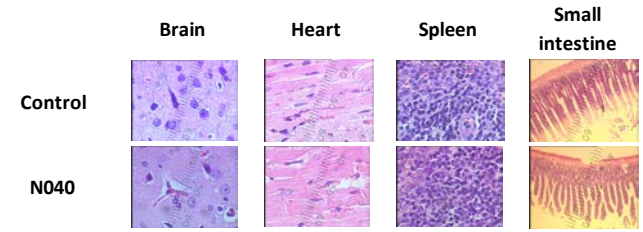


Figure 5 Histopathology of brain, heart, spleen and small intestine of rats in the sub-chronic toxicity study at the end of the study (90 days) fed with the recipe N040 extract (1,000 mg/kg body weight per day, p.o.).

Preparation of N040 capsules

The N040 recipe extract was developed as capsules. The extract was prepared in granules and filled in capsules by weighing the extract, and mixing with other additives. The wet granules were prepared and passed through a sieve, and dried in an oven. The dried granules were passed through a sieve. The granules were filled in capsules. The appearances of granules and capsules of the N040 recipe extract were shown in **(Figure 6)**.

Figure 6 The appearances of granules and capsules of the N040 recipe extract

Conclusion

This developed capsules containing the N040 recipe extract selected from the Manosroi III database which were preclinically proved to have effective anti- cervical cancer activity and safe use in animal tests are now in the process of clinical test in the cervical cancer patients who do not response to the standard treatment, by the Department for Development of Thai Traditional and Alternative Medicine, Ministry of Public Health in Thailand.

Acknowledgements

The investigators would like to thank the Institute of Thai Traditional Medicine, Ministry of Public Health, Nonthaburi, Thailand for the financial supports of this study.

The pre-clinical research of anti-cancer Thai medicinal plant recipe N040 for the further investigation in advanced cervical cancer patients not responded to the standard treatment

Supported by :

- The Institute of Thai Traditional Medicine, Ministry of Public Health, Nonthaburi, Thailand

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