Potent anti-cervical cancer activity of Thai medicinal plants recipe (MANOSE N040)
selected from the MANOSROI III database

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Cervical cancer is the second most common malignancy for women after breast cancer.

Approximately 83% of the new cases and 85% of deaths from cervical cancer have been reported in the developing countries.

More than 75% of the cases in these countries were diagnosed as advanced stages.¹)

Thai medicinal plants

- In Thailand, both single and mixed medicinal plants in the forms of recipes have been widely used for the treatment of many diseases for several generations.
- The evidences of usage medicinal plants were Thai medicinal plant textbooks, palm leaves and *Streblus asper* paper.\(^2\)

The database “MANOSROI III”

A large number of recipes from all regions of Thailand have been collected, translated and put in the MANOSROI III database.

At present, MANOSROI III database contains 83,000 recipes out of 200,000 expected recipes covering several diseases including cancer.

MANOSROI III database contains total of 723 anti-cancer recipes.
Thai medicinal plant recipe N040

- The N040 Thai medicinal plant recipe from the Manosrooi 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

<table>
<thead>
<tr>
<th>Recipe no.</th>
<th>Source</th>
<th>Preparation</th>
<th>Example of plants compositions in the recipe</th>
</tr>
</thead>
</table>
| 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*  
*Polyalthia debilis*  
*Nymphoides indicum*  
*Psophocarpus tetragonolobus* |
Example of plants compositions in Thai medicinal plant recipe N040

*Urceola minutiflora*

*Sida rhombifolia*

*Polyalthia debilis*

*Nymphoides indicum*

*Psophocarpus tetragonolobus*
Extraction of 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, 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.
Thai medicinal plant recipe N040 extract have been investigated the *in vitro* and *in vivo* anti-cervical cancer activity.

Cell line was used in this study

Human cervical adenocarcinoma (HeLa)
Part I: Investigation of *in vitro* anti-cervical cancer activity of recipe N040

The extract of recipe N040

- Anti-proliferative activity by sulforhodamine B (SRB) assay
- Apoptosis induction
  - Acridine orange (AO) and ethidium bromide (EB) staining
  - Caspase-3 activity assay

Part II: Investigation of *in vivo* anti-cervical cancer activity of recipe N040

The extract of recipe N040

*In vivo* anti-cervical cancer activity using HeLa cell xenograft nude mice model\(^4\)

Subchronic toxicity in rats\(^5\)

Nude (BALB/cMalc-nu) mice (Mus musculus)

Wistar rat (Rattus norvegicus)

Implanted HeLa cell line (5.0 x 10^6 cell) in the right flank region of nude mice (BALB/cMlac-nu) using subcutaneous injection.

Started treatment when tumor volume was 80-100 mm^3

The mice were divided into five groups (3 mice per group) as follows:
- Low dose group (N040 8.90 mg/kg BW/day)
- Medium dose group (N040 44.50 mg/kg BW/day)
- High dose group (N040 89.20 mg/kg BW/day)
- Control group (Sterile normal saline)
- Positive control group (Cisplatin 5 mg/kg BW/week was given by i.p.)

Given by orally administered

The mice were treated for 14 days and measured tumor volume using vernier caliper everyday for evaluate anti-tumor activity.

At the end of treatment, the mice were sacrificed and excised tumors.

The tumors were weighed individually.

Tumor volume (T_v) : \[ T_v = \frac{a^2 \times b}{2} \]
when
- a referred to the shorter dimension (mm)
- b referred to the longer dimension (mm)

The rats were divided into two groups (10 male and female rats per group) as follows.  
- **Treatment group** (N040 1,000 mg/kg BW/day) 
- **Control group** (Sterile normal saline) 

The rats were treated for 90 days and observed the apparent signs of toxicity and behavior alteration during the experiment.

At the end of treatment, blood was collected for **hematology and clinical biochemistry** tests.

The animals were sacrificed and examined for **histopathology of organs** (brain, heart, lung, liver, kidney, spleen, stomach, intestines and gonads).

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Results

**Part I: Investigation of *in vitro* anti-cervical cancer activity of recipe N040**

1.1 Anti-proliferative activity using 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 The IC$_{50}$ values (µg/ml) of the highest activity of the extracts on human cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Recipe no.</th>
<th>IC$_{50}$ values (µg/ml)</th>
<th>Folds of anti-cancer standard drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>HeLa</td>
<td>N040</td>
<td>0.11±0.03</td>
<td>24.91</td>
</tr>
</tbody>
</table>

Note: N = North region and
1.2 Apoptosis induction using acridine orange (AO) and ethidium bromide (EB) staining

![Image of green cells with annotations]

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

**Note:** Normal green represents healthy cell (H). Bright green represents an apoptotic cell (A). Orange represents late apoptotic cells with secondary necrosis (N).

**Table 3** The % apoptotic cell of the highest activity of the extracts on human cancer cell lines at IC50 values concentration

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Recipe no.</th>
<th>% apoptotic cell</th>
<th>Folds of anti-cancer standard drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>HeLa</td>
<td>N040</td>
<td>1.35±1.18</td>
<td>0.18</td>
</tr>
</tbody>
</table>
1.3 Apoptosis induction using caspase-3 activity assay

The N040 recipe extract gave the apoptosis activity of HeLa cancer cell line via caspase-3 induction (Table 4).

**Table 4** The Folds of control of the highest activity of the extracts on human cancer cell lines at IC50 values concentration

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Recipe no.</th>
<th>Folds of control</th>
<th>Folds of anti-cancer standard drug</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>HeLa</td>
<td>N040</td>
<td>1.21</td>
<td>1.06</td>
</tr>
</tbody>
</table>
2.1 HeLa cell xenograft nude mice model

The relative tumor volume of HeLa xenograft nude mice model fed with the recipe N040 extract (low dose: 8.9, medium dose: 44.50 and high dose: 89.20 mg/kg BW/day) for 14 days.

Relative tumor volume (RTV) : \[ RTV = \frac{V_t}{V_0} \]
when \[ V_0 \] referred to the tumor volume at day 0
\[ V_t \] referred to the tumor volume at day t

Note: * significantly \((p<0.05)\) different than that of control

The N040 extract at the medium dose and cisplatin significantly \((p<0.05)\) inhibited the tumor growth with the relative tumor volume of 0.55 and 0.18 fold the control, respectively.

There were no mortality, weight loss and noticeable major side effects of the animals treated with the extract at all doses of the N040 extracts and cisplatin.
Figure 2 Sizes of tumors on nude mice at the end of treatment

- **N040 low dose** (8.90 mg/kg BW/day)
- **N040 medium dose** (44.50 mg/kg BW/day)
- **N040 high dose** (89.20 mg/kg BW/day)
- **Control (normal saline)**
- **Cisplatin** (5 mg/kg BW/week)
### Table 5  The relative tumor weights and the percentages inhibition of relative tumor weight of HeLa xenograft nude mice model fed with the recipe N040 for 14 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Relative tumor weight (g)</th>
<th>% inhibition of relative tumor weight</th>
<th>Folds of Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.78±0.24</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Low dose</td>
<td>2.52±0.39</td>
<td>33.33</td>
<td>0.38</td>
</tr>
<tr>
<td>Medium dose</td>
<td>1.62±0.24</td>
<td>57.23</td>
<td>0.65</td>
</tr>
<tr>
<td>High dose</td>
<td>1.94±0.28</td>
<td>48.67</td>
<td>0.55</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>1.55±0.97</td>
<td>87.83</td>
<td>1.00</td>
</tr>
</tbody>
</table>

The N040 extract at the medium dose (44.50 mg/kg BW/day) gave the highest percentage inhibition (57.23 %) which was 0.65 fold of cisplatin (87.83 %).

\[
\text{% inhibition of tumor weight} = \left(\frac{\text{RTW}_c - \text{RTW}_{\text{treat}}}{\text{RTW}_c}\right) \times 100
\]

when \(\text{RTW}_c\) referred to the relative tumor weight of the control mice

\(\text{RTW}_{\text{treat}}\) referred to the relative tumor weight of the treated mice.
Histopathology of tumor mass in control and N040 low dose group

Histopathology of tumor mass in cisplatin group

Histopathology of tumor mass in N040 medium and high dose group

The atrophy of the cancer cells with fragmented nucleus indicating of apoptosis was observed.
### 2.2 Subchronic toxicity in rats

**Table 6 Subchronic toxicity study in rats** treated with the recipe N040 extract (1000 mg/kg body weight per day, p.o.) at the end of the study (90 days)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight gain</th>
<th>Hematology</th>
<th>Clinical blood chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver and kidney function tests</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>N040</td>
<td>Normal</td>
<td>MCH increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>N040</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Note:** MCH = Mean corpuscular hemoglobin
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control male</th>
<th>Treated male</th>
<th>Control female</th>
<th>Treated female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (40X)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Liver (40X)</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>Kidney (40X)</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
<tr>
<td>Small intestine</td>
<td><img src="image13.png" alt="Image" /></td>
<td><img src="image14.png" alt="Image" /></td>
<td><img src="image15.png" alt="Image" /></td>
<td><img src="image16.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 3** HE staining of lung, liver, kidney and small intestine of rats in the subchronic toxicity study at the end of the study (90 days) fed with the recipe N040 extract.

The minor toxicity of the extract might be due to the high dose (1,000 mg/kg body weight per day) of N040 used in the study, which was about 112 folds of the normal traditional dose (8.90 mg/kg body weight per day) and about 22 folds higher than the effective dose (44.50 mg/kg body weight per day) in HeLa cell xenograft nude mice model. These results indicated the safety of N040 recipe.
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 4).

Figure 4 The appearances of granules and capsules of the N040 recipe extract
Conclusion

• This study has demonstrated the potent *in vitro* and *in vivo* anti-cervical cancer activity and confirmed the traditionally use of the recipe N040 selected from the Thai/Lanna medicinal plant recipe database “MANOSROI III” with the potential for the further development of this recipe to novel anti-cervical cancer drugs.

• This developed capsules containing the N040 recipe extract 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.
Acknowledgement

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Manose Health and Beauty Research Center, Chiang Mai, Thailand
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