3 Promoting herbal drugs: Thailand

General information

- **Implementing institution**
  Chulabhorn Research Institute (CRI)
- **Head**
  Professor Dr. HRH Princess Chulabhorn (president)
- **Details of institution**
  Address: Chulabhorn Research Institute (CRI), Vipavadee Rangsit Highway, Laksi, Bangkok 10210, Thailand

  Tel.: (+66) 2 574 0615 / 0622 33 ext. 1610

  Fax: (+66) 2 574 0616 / 575 1497

  E-mail: ac@tubtim.cri.or.th

  Web site: www.cri.or.th
- **Implementation period**
  Feasibility study: 1999. Project: 2000-2002
- **Costs**
  Funds were provided by the United Nations Development Programme (UNDP): US$76,500, and CRI: US$2,200,000.

SUMMARY

Between January 2000 and December 2002, the Chulabhorn Research Institute (CRI) received funding from the United Nations Development Programme (UNDP) to implement a project entitled "Green Health Technology for Women's Empowerment and Sustainable Development". Among other components, the project aimed to develop the use of two medicinal plants (*Curcuma longa* and *Andrographis paniculata*) for simple illnesses and to promote the cultivation of these plants as a source of additional income. This case study, which illustrates the integration of field and laboratory activities to the benefit of all parties, focuses on *A. paniculata* (in Thai, "fah thalai jone").

It is generally accepted that, in the case of both modern drugs and herbal drugs, therapeutic efficacy and safety must be proven. In herbal drugs, variations in the levels of active ingredients are well documented and can account for therapeutic ineffectiveness and side effects. Therefore, to promote the use of herbal drugs, scientific intervention is necessary at all steps, starting from good agricultural practices and continuing through good manufacturing practices.

The project undertaken by CRI covered both field and laboratory activities in parallel. CRI scientists successfully isolated three major active chemicals from *A. paniculata* — the diterpenoids andrographolide (AP_1), 14-deoxy - 11,12-didehydroandrographolide (AP_3) and neoandrographolide (AP_4) — and studied their pharmacological and toxicological activities. In addition, a simple and rapid method using high performance liquid chromatography (HPLC) to analyse these three major chemicals simultaneously was successfully developed. Indeed, HPLC was used throughout the project, for example:

- to check the quality of the finished *A. paniculata* powdered and extracted products;
- to study the stability of the *A. paniculata* products; and
- to select the appropriate cultivation period to obtain the maximum levels of the desired active compounds.

One of the few side effects observed in some patients using *A. paniculata* products was the lowering of blood pressure. AP_3 was identified as the compound that induced this antihypertensive effect, and it was also found that levels of AP_3 in products increased during storage. Therefore, to avoid this side effect, it is advised that crude *A. paniculata* powdered products be used within one year. Attempts are also being made to determine the cultivation conditions that result in high levels of AP_3 and to study the potential of AP_3 as an antihypertensive drug.

In summary, this project has achieved the following:

- identification of the appropriate conditions for the cultivation and harvesting of *A. paniculata*;

- isolation of the pure active diterpenoids;
- development of a rapid and simple HPLC method to analyse three bioactive diterpenoids simultaneously;
- identification of bioactive compounds that cause a lowering of blood pressure;
- estimation of the shelf life of crude *A. paniculata* powdered drug;
- identification, in preliminary studies using water extracts, partially purified extracts and pure diterpenoids, of antimalarial activity and antiplatelet aggregation; and
- generation of supplementary income for women participating in the project.

Background and justification

The ultimate goal of the Chulabhorn Research Institute (CRI) is to use science and technology to improve people's quality of life.

After the successful organization of the Princess Chulabhorn Science Congress in 1999, His Majesty King Bhumibol Adulyadej advised HRH Princess Chulabhorn, president of CRI, to develop a project that would benefit people at the grass-roots level.

In response, HRH Princess Chulabhorn held an executive meeting to formulate the project. During this period, CRI was in the process of preparing a project proposal seeking support from UNDP. Therefore, it was decided that the Institute would attempt to use science and technology to improve the quality of life of the people not only through the use of medicinal plants to treat ailments but also through their cultivation to provide a supplementary income for poor families in rural areas.

Since 1980, Thailand's Office of Primary Health Care in the Ministry of Public Health has classified herbal drugs as "essential drugs" that could be used among the strategies to achieve "Health for all by the year 2000".

In the last decade, there has also been a resurgence of interest in herbal medicines in Western societies. With the rapid increase of market demand, cultivation of medicinal plants is obviously needed, especially as many medicinal plants continue to be gathered from wild sources.

The first volume of the Thai Herbal Pharmacopoeia, published in 1995, lists specifications and related data concerning the quality of herbal drugs. In 1999, the National Essential Drug List Committee of the Ministry of Public Health released the "National List of Essential Drugs: List of Herbal Medicinal Products". *Curcuma longa* and *Andrographis paniculata* were included on this list.

C. longa (turmeric, family Zingiberaceae) was selected because of its wide range of uses in food, cosmetics and health care. The variety that gave the highest yields of the

desired volatile oils and curcuminoids was selected for cultivation. The rhizomes are sold as raw materials to the local drug industry.

A. paniculata (family Acanthaceae) was selected because it has been widely used to treat the common cold. From personal communications with practitioners of traditional medicine, it was discovered that, in some patients, this plant caused a fall in blood pressure. The scientific literature also shows that *A. paniculata* has a wide spectrum of pharmacological activities, including antidiarrhoeal, antihepatotoxicity, antimalarial and antiviral (including anti-HIV) activities. It is also active against the common cold and has immunostimulant and cardiovascular activities.

At present, more than 20 *A. paniculata* preparations are available on the local market. Among these, very few are manufactured by using good manufacturing practices. Although the total levels of the active ingredients (diterpenoid lactones) in *A. paniculata* from different sources are similar, the proportion of each varies depending on the season and time of harvest (fig. 1). To develop a standardized testing procedure, biomarkers for the biologically active compounds are needed. Ideally, a simple and rapid analytical technique should be developed that can be used in the quality control of both the raw materials and finished products and as a means to study the shelf life of products to ensure their safety and therapeutic effectiveness.

The project was aimed at assisting both local communities in producing quality raw materials to supplement their income and local manufacturers in producing quality products through the application of science and technology. In addition, the isolated bioactive compounds are being used for further pharmacological and toxicological studies.

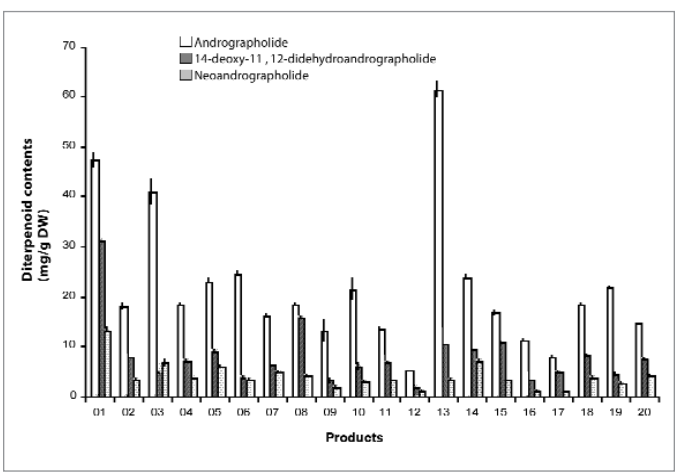

Figure 1 |
Variation of andrographolide (AP_1), 14-deoxy-11,12-didehydro- andrographolide (AP_3) and neoandrographolide (AP_4) contents in *A. paniculata* products from different suppliers in Thailand. Values represent the mean of three replicates with standard error of means. *(Reproduced by permission of John Wiley and Sons Ltd.)*

Description

The project was aimed at applying sophisticated science and technology to benefit the rural community. Two major complementary activities were conducted in parallel, namely, practical activities at the rural community level and scientific and technical activities in CRI laboratories.

Before starting the project, a feasibility study helped to select the project site, Tubtim Siam Village 5, Srakaew Province, near the Cambodian border, and the participating women. The selection of the women, 35 in all, was based on their economic status, level of education and willingness to participate in the project.

A project committee was appointed and key personnel were identified. A coordinating group comprised core staff from CRI. This group invited a team of experts from the Faculty of Pharmacy, Mahidol University, Thailand, to conduct a training course on cultivation with good agricultural practice (GAP) during the first year of the project. Later, the monitoring and supervision were carried out by a team from CRI, who reported the results directly to the coordinating group.

Cultivation and processing of medicinal plants

The area to be cultivated was improved by prior ploughing and the planting of nitrogen-fixing legumes. No chemical fertilizers were used.

One to three *A. paniculata* seeds were planted in pots containing a 1:1 mixture of soil with either leaf mould, peat moss or manure with peat moss. Approximately 45 days later, seedlings were transplanted into the cultivation area. After three months, robust leafy plant growth was seen. At the fourth month, about 50 per cent of the plants were flowering and the plants were deemed ready for harvest (fig. 2). Laboratory analyses showed that the three active ingredients were present at higher levels in the leaves than the stems and that the optimum time for harvesting was just before flowering.

For the first two crops, leaves were gathered by hand, air dried, then oven dried at approximately 50°C to a constant weight, weighed and packed. However, since this was labour intensive, an alternative method was introduced, involving hand cutting of the whole plant at ground level. Fresh or dried leaves were removed from the whole plants using a rotary agitator and a forced air current was used to blow the leaves into a collecting bin. This equipment was built and operated at the project site.

Part of the processed medicinal plant material was used by the CRI laboratories, while the remainder was sold to a traditional medicine outlet for profit. Good-quality dried leaves of *A. paniculata* could be sold for US$5 per kilogramme, while purified active ingredients, andrographolide and its derivatives, cost as much as US$100,000 per kilogramme from specialist chemical suppliers. CRI calculations based on the estimated content of these pure compounds in *A. paniculata* leaves predict that, in the future, it

will be more profitable to purify the active bioactive chemicals for sale.

Laboratory activities

The scientific activities carried out at CRI laboratories included:

- initial soil and water analyses to determine optimal cultivation-site characteristics;
- analysis of soil and water by atomic absorption spectrometric methods to ensure that levels of heavy metal contaminants in the soil and water did not exceed safe limits;
- analysis of soil and water by gas chromatography to check for contaminating pesticides;
- determination of optimum conditions for seed germination, planting time, growth period and harvest time to maximize yields of the bioactive chemicals;
- optimization of the techniques for isolating, purifying and characterizing the structure of the diterpenoids (andrographolide and its derivatives), the bioactive compounds in *A. paniculata*, using HPLC, nuclear magnetic resonance imaging and mass spectrometry. Purified active compounds were used as standards in HPLC analyses;
- assays of the pharmacological properties of partially purified extracts and pure compounds isolated from *A. paniculata*; and
- development of a rapid and simple HPLC method to quantify simultaneously the three major active compounds: andrographolide (AP_1), 14-deoxy-11,12-didehydroandrographolide (AP_3) and neoandrographolide (AP_4).

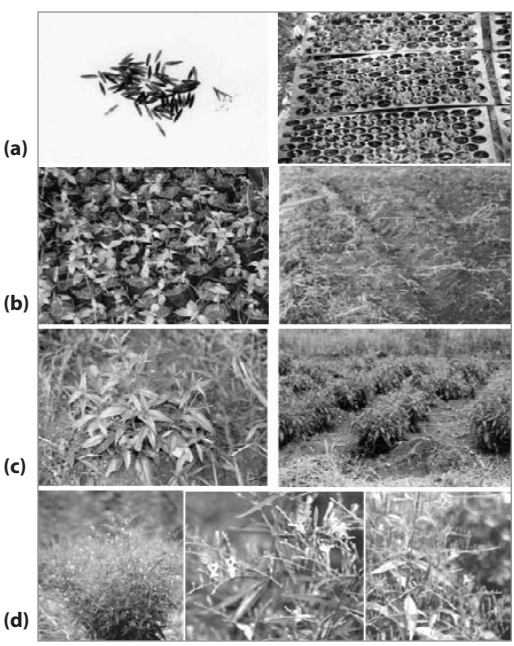

Figure 2 | Cultivation of *Andrographis paniculata*. Row A: seed germination; B: 45-60 days after planting; C: 3 months after planting; D: flowering plants. *(Reproduced by permission of John Wiley and Sons Ltd.)*

Among the pharmacological properties of the diterpenoid compounds assayed were their effects on the cardiovascular system.

It was demonstrated that AP_3 significantly decreased the beating rate of right atria isolated from the hearts of rats. AP_3 also ameliorated vasoconstriction induced by norepinephrine in isolated descending aortas of rats more strongly than AP_1, while the application of AP_4 had little or no effect. Considering the levels of active ingredients in the recommended daily dose of various commercial products, certain products (fig. 1, Nos. 1, 5, 8, 13, 14 and 15) contained elevated levels of AP_3, which may account for the observed effects on the blood pressure.

In other studies, dried powder of *A. paniculata* harvested at different times in 2001 (18, 25 and 26 July and 8 August) were kept in plastic bags and stored at room temperature (approximately 28° to 30°C). After 0, 3, 6, 11, 12 and 15 months, samples were extracted and analysed for the contents of the three diterpenoid active ingredients (AP_1, AP_3 and AP_4) using the HPLC analytic method developed by CRI laboratories. It should be noted that the contents of AP_3 in all samples tested increased with storage time while the two other active compounds (AP_1 and AP_4) decreased over time (fig. 3). These results suggest that the stability of these active compounds should be taken into consideration when this herb is stored for a period to ensure the efficacy and safety of the herbal products.

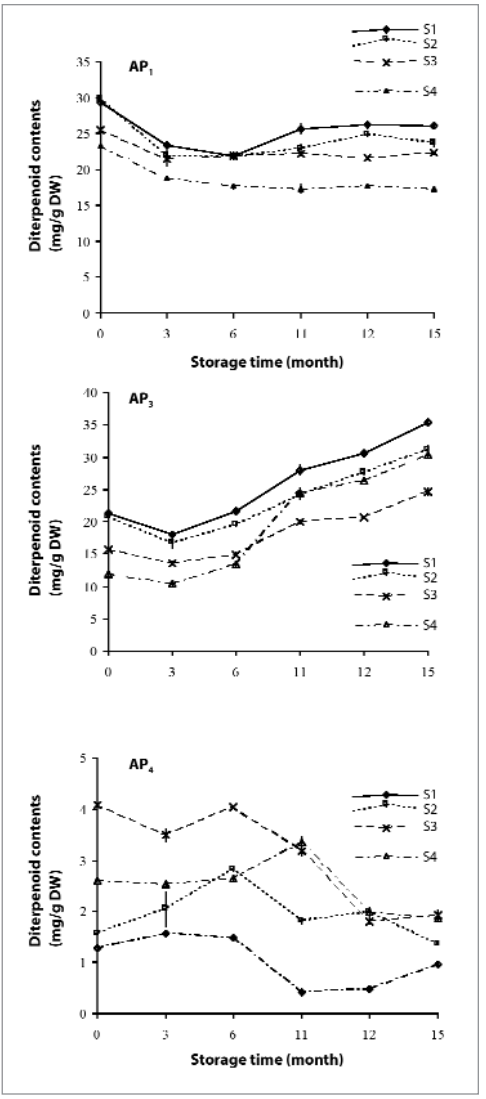

Figure 3 | Changes in andrographolide (AP_1), 14-deoxy-11,12-didehydroandrographolide (AP_3) and neoandrographolide (AP_4) contents in dried *A. paniculata* stored for up to 15 months at room temperature. (Values represent the mean of three replicates with standard error of means.) Samples S_1, S_2, S_3 and S_4 were harvested on 18 July, 25 July, 26 July and 8 August 2001, respectively. *(Reproduced by permission of John Wiley and Sons Ltd.)*

Patenting and commercialization

Recently, pure compounds have been isolated and these will be made commercially available. The analytical method and the process of preparing *A. paniculata* extracts with different proportions of AP_1, AP_3 and AP_4 is being patented.

Partnerships

The project was partly funded by UNDP.

The project needed the skills of a wide variety of experts, including economists, botanists, agriculturists, pharmacists, phytochemists, pharmacologists and toxicologists. In addition, it required the close collaboration of the local community, academic and industry groups. Good cooperation among all partners was essential, and many obstacles were overcome through the efforts of the involved parties because this was a project initiated by the Royal Family.

However, in the beginning, links with the private sector based on trading the raw materials were not satisfactory because each party wanted the greatest benefit for itself. However, in the second, ongoing phase of the project, which involved developing the herbal drugs for poultry (see Impact below), links between the private sector and CRI were strengthened because both parties had the common goal of wanting to assist poor farmers.

Replicability

Medicinal plants have been used for centuries in many parts of the world and their proper application has been practised by knowledgeable traditional doctors. However, in several countries, mass deforestation is leading to a decline in the popularity of medicinal plants. The revitalization of the use of medicinal plants demands both an adequate and stable supply of the species concerned and an accurate knowledge of their traditional uses. The techniques used to validate the traditional knowledge of the medicinal plants used in this project can easily be applied to other medicinal plants in other regions. However, such projects must be appropriately supported and have access to well-equipped laboratories.

Policy implications

The study was initiated in response to the policy of the Government of Thailand to promote the use of herbal drugs and the policy of CRI to use science and technology to improve the quality of life of the people. Likewise, the success of similar projects will need clear policies to apply the lessons learned from this project in the development of pharmaceutical products from selected medicinal plants.

To date, the study has not resulted in any changes in legislation. However, a recommendation for the quality control of *A. paniculata* products can be suggested. At present, the quality standards for *A.*

paniculata are set by the Ministry of Public Health and state that the total lactone content calculated as andrographolide must not be less than 6.0 per cent; a simple method for the analysis of total lactone is also described. The results of this study suggest that the total lactone content is not an appropriate marker, since the total lactone content of two preparations may not be significantly different, but the content of each bioactive diterpenoid may be markedly different, which will affect the therapeutic effectiveness and side effects of *A. paniculata* preparations. Therefore, the result of this study will be submitted to the Ministry of Public Health for consideration in modifying the standards of this medicinal plant for specific therapeutic indications. In addition, *A. paniculata* preparations used for a sore throat should have standards relating to the shelf life of the preparation. In particular, it is recommended that preparations be used within one year in order to avoid side effects related to the lowering of blood pressure.

Lessons learned

The objective of the project was to use science and technology to improve the quality of life of the poor and to discover how to transfer the knowledge from laboratory work for practical uses to the people who not only have no background in science but also poor literacy. The women participating in this project had a low educational background and income.

Initially, it was difficult to convince the women to follow the strict instructions for good manufacturing practices. However, using suitable demonstrations, the women could be taught to avoid certain poor practices. For example, one batch of *C. longa* rhizomes was rejected because of bacterial contamination. A simple microscope was taken from the CRI laboratories and used in a one-day training course about microorganisms. Each participant was given the opportunity to see the bacteria under the microscope. After this, they were more willing to follow the instructions.

The labour required to produce raw materials for herbal drugs using good agricultural practices (for example, plots are hand-weeded and leaves are picked by hand) meant that the dried leaves were intended to be sold at US$5 per kilogramme. However, herbal drug manufacturers still preferred cheaper-to-produce, lower-grade raw materials. To solve this problem, studies of seed germination at various times of the year were conducted so that *A. paniculata* could be cultivated according to market demand. In addition, the four largest herbal drug manufacturers were invited to inspect the raw materials and to watch a videotape showing the good agricultural practices. They were also asked to place their orders for raw materials about four months in advance. However, this approach was not fully successful because the higher price of the raw materials would have meant raising the price of the commercial products.

Unsold *A. paniculata*, therefore, was used as a raw material for isolating pure biologically active compounds. The catalogue price of andrographolide is US$61.50 for 500 milligrammes, equivalent to US$123,000 per kilogramme. Several pure compounds were isolated and are being used as standard drugs for the Institute's internal use. They are also being distributed at cost to other government agencies upon request.

IMPACT

Demand for herbal drugs is increasing and maintaining an adequate supply from wild sources is not sustainable. The cultivation of medicinal plants can be sustainable and environmentally sound but, to be economically viable, plants may have to be produced outside their natural season.

A. paniculata grows during the rainy season but, to prevent over supply, it should be cultivated throughout the year according to demand. Doing so, however, results in varying concentrations of active ingredients in the leaves. Such variation can be detected and standardized by using the simple and rapid analytical method that has been developed by CRI scientists. This innovation makes production of the herbal drug sustainable and economical. The analytical method will be published in an international journal and will benefit everyone working with this medicinal plant.

The completion of the first phase of this project coincided with the urgent need of the country to develop herbal drugs for poultry. Antibiotic residues in chicken have posed a serious problem for the Thai economy as contaminated products have been rejected for export purposes. Therefore, the use of most antibiotics has been banned and alternative drugs for poultry, including those derived from medicinal plants, represent an urgent national need. Knowledge of the appropriate cultivation of *A. paniculata* and the development of analytical techniques to quantify its three bioactive compounds are very useful for the further development of this herbal drug for use in poultry. The development of pharmaceutical products for poultry based on A. *paniculata* has therefore been initiated in collaboration with Tanaosri Farm in Rajaburi Province, Thailand.

A representative of CRI was invited to participate in a closed meeting of academia, industry and government agencies about developing the use of medicinal plants, including *A. paniculata*, for domestic animals. CRI has expressed its willingness to cooperate with the private sector. Recent outbreaks of bird flu in Thailand have also increased public awareness, through local newspapers and television, that traditional knowledge of medicinal plants can be harnessed for treating diseases in animals.

In addition, the identification of AP_3 as the most active chemical for lowering blood pressure is important, especially when it was discovered that levels of this compound increased during storage. Not only is this information useful for avoid-

ing side effects, but isolation of this compound for its use as a hypertensive drug may also be beneficial and further studies are ongoing.

FUTURE PLANS

A study to determine how the levels of each active compound in *A. paniculata* change during the growth of the plant has been initiated. The results will facilitate the isolation of pure active compounds that can then be sold as standard chemicals.

At present, most *A. paniculata* products available in the market are in the form of a crude dried powder from leaves and stems. The modification of such drug preparations in the form of capsules containing standardized doses of extract will improve the quality of the products.

There are also plans to begin large-scale purification of biologically active chemicals, as these compounds should prove useful in the quality control of herbal drugs. Further studies on the pharmacological and toxicological effects, which will lead to the development of modern drugs, will also be conducted.

At present, even though the analogues of several purified chemicals have been synthesized and patented by a leading drug company, these products will be costly. Therefore, it is anticipated that herbal preparations will still be required in developing countries. Since the yield of andrographolide is quite high and the processes of extraction, separation, isolation and purification have been determined, production in the form of standardized extracts from the natural source will be inexpensive. Furthermore, its production should generate additional income for poor farmers.

CRI also plans to offer a quality-control service for local *A. paniculata* products at a minimal charge.

PUBLICATIONS

Pholphana, N., Rangkadirok, N., Thongnest, S., Ruchirawat, S., Ruchirawat, M., and Satayavivad, J. Determination and variation of three active diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. Phytochemical Analysis. (In press)

Sahasitiwat, S. (2002). The study of acute cardiovascular toxicity of diterpenoid lactones isolated from *Andrographis paniculata* (Burm.f.) Nees. M.Sc. (Toxicology) Thesis, Faculty of Science, Mahidol University, Thailand (ISBN 974-04-2008-7).

A videotape on the "Green Health Technology for Women's Empowerment and Sustainable Development" project has been prepared and is available on request.

Case study prepared by:

Jutamaad Satayavivad
Office of Academic Affairs, Chulabhorn Research Institute, Vipavadee Rangsit Highway, Laksi, Bangkok 10210, Thailand
E-mail: jutamaad@tubtim.cri.or.th

Project participants:

HRH Princess Chulabhorn: Set the policy of the Institute to conduct a project that could apply science and technology to benefit people at the grass-roots level.

Mathuros Ruchirawat, Vice President for Research: Implemented and coordinated the project.

Somsak Ruchirawat, Associate Vice President for Research: Phytochemical studies of the selected medicinal plant products.

Jutamaad Satayavivad, Associate Vice President for Academic Affairs: Responsible for field and laboratory studies of the medicinal plant products.

Project consultants:

Feasibility study for selected medicinal plants and field activity (Faculty of Pharmacy, Mahidol University):

Promjit Saralamp, Department of Pharmaceutical Botany.

Noppamas Soonthornchareonnon, Department of Pharmacognosy.

Sompop Prathanturarug, Department of Pharmaceutical Botany

Project site selection:

Damrong Ratanapanich, Vice President for Special Activities, CRI.

CRI staff:

Coordinating staff and field work:

Roengwit Phetkaochouy, Krittika Polratana, Sujunya Ruengverayut, Wandee Sirapat.

Phytochemical group:

Vanida Bhavakul, Piyanun Boonprasert, Sanit Thongnest.

Pharmacological group:

Nuchanart Rangkadilok, Jittra Hun, Sumontha Nookabkaew, Nanthanit Pholphana, Sumitra Suntararuks, Luksamee Worasuttayangkurn.