**Introduction**

*Stephania venosa* Spreng (*S. venosa*), commonly known in Thai as Boraphet-phungchang, Sabu-le-ad or Kling klaang, is a plant in the family of Menispermaceae. Many parts of this plant had been used as a folk remedy for multiple purposes such as stem for anthelmintic, rhizome for neuronal function, leaf for wound healing, and tuber for food appetite and health. Many studies have reported that *S. venosa* tuber in Thailand revealed a variety of pharmacological activities including antimalarial, acetylcholinesterase inhibitors\(^2\) antiproliferative activity on cancer cells\(^3,4\) and anti-inflammatory activity.\(^5\) Over thirty isoquinoline alkaloids in the tuber have been identified\(^6-8\) and demonstrated many biological activities, such as aromoline possessed anti-plasmodial, antiamoebic and cytotoxic activities; berbamunine and tetradine could inhibit production of interleukin-1 and tumour necrosis factor; berbamunine and cepharanthine displayed anti-inflammatory and immunosuppressive activities; dicentrine showed antihypertensive activity; and palmatine had sedative effect.\(^9,10\)

Although most people have used the tuber of *S. venosa* in the form of crude drugs, boiled solution, liquor soaking or pills, the effectiveness of these formulations have not been scientifically verified. Our previous observation found that animals orally taking water extract from the tuber of *S. venosa* become sedated. Sedative activity of any compounds may involve inhibition of excitatory neurotransmitter. Serotonin (5-HT) acts as an excitatory neurotransmitter when it binds to 5-HT\(_2\) receptor subtype. Many studies suggest that the expressed 5-HT receptor in *Xenopus* oocyte after injection of rat brain mRNA is 5-HT\(_2\)-
like receptor. Therefore, this study aimed to evaluate the
effects of water extract from S. venosa tubers on 5-HT
receptor using the model of Xenopus oocyte.

Materials and Method

Plant material and extract preparation

Authentication of the tuber of S. venosa (Bl.) Spreng was
achieved by comparison with herbarium specimens at the
Bangkok Forest Herbarium, Royal Forest Department,
Ministry of Agriculture and Cooperatives, Bangkok, Thailand.
S. venosa tubers were collected from Rachaburi Province,
Thailand. The fresh tubers were harvested, chopped into
small pieces and dried under the sun light for 2 - 3 days.
The dried S. venosa tubers were boiled for 5 h. The
solutions were then filtered and lyophilized using a freeze
dryer to yield a brown powder.

Oocyte injection

Defolliculated stage V–VI oocytes were prepared from
Xenopus laevis (Xenopus Express, Cape, South Africa) as
described previously 11. Briefly, Xenopus laevis were
anesthetized in ice-water, and a lobe of the ovary was
dissected and placed in sterile modified Barth’s solution
(MBS: 88 mM NaCl, 1 mM KCl, 0.41 mM CaCl2, 0.33 mM
Ca(NO3)2, 0.82 mM MgSO4, 2.4 mM NaHCO3, 7.5 mM Tris
(hydroxymethyl) aminomethane, pH 7.6). Oocytes were then
isolated manually and defolliculated by incubation in 2 mg/ml
collagenase (type IA; Sigma, St. Louis, MO, USA) at 19 °C
for 1 h in calcium-free MBS solution. To examine 5-HT or
glycine receptor function, total mRNA was prepared from
whole brain or spinal cord of adult male rats, respectively,
using the Trizol reagent (Gibco-BRL) 12, and oocytes were
injected with 46 nl of the total mRNA (5 mg/ml). To examine
NMDA receptor function, cRNA was prepared from cDNA
clones of NR1a and NR2B kindly provided by Dr. K. Igarashi
(Faculty of Pharmaceutical Sciences, Chiba University) and
oocytes were injected with 27.6 nl of NR1a/NR2B RNA
mixture 13. After injection, oocytes were incubated in MBS
containing 2.5 units /ml penicillin and 2.5 μg/ml streptomycin
at 18 °C.

Electrophysiological recording

Responses to 5-HT were recorded using a two-electrode
voltage-clamp amplifier (GeneClamp 500B; Axon Instrument,
Foster City, CA, USA) at a holding potential of -70 mV
unless noted otherwise. Electrodes were filled with 3 M KCl
and had resistances of 0.5 – 5 MΩ. Oocytes were
positioned in a 50-μl chamber and continuously perfused
with MBS solution at 1 ml/min at room temperature. The
drugs were applied until a plateau or peak of the response
was observed. Data were recorded and digitized for analysis
(MacLab 200; AIDInstruments, Castle Hill, NSW, Australia).
The washout period for recovery was 3 – 5 min, depending
on the concentration of drugs applied. Most data was
expressed as the mean ± S.E.M. For statistical analysis, the
SigmaStat (ver 3.5) program was used. Data were subject to
the paired t-test when effects were compared between
before and after drug application in the same oocytes or the
unpaired t-test when the experiments were performed in
different oocytes. If the data were compared with 100%
control, Mann-Whitney Rank Sum test was used. Differences
with P < 0.05 were considered significant.

Results

Dose-dependent inhibition of S. venosa extract on 5-HT
receptors

To examine inhibitory effect of the extract on the
expressed 5-HT receptors, 0.1 μM 5-HT was applied to
oocytes in the absence and presence of the extract.
Simultaneous application of the extract at 10, 100 and 1000
μg/ml and 5-HT to oocytes produced 9 ± 7.7%, 65 ± 5.5%
and 80 ± 2.3% inhibition, respectively (Fig. 1A, 1C).
Pretreatment of oocytes with the extract for 1 min before
simultaneous application, the inhibition were 25 ± 3.4%, 75 ±
7.7% and 100 ± 0% for the extract at 1, 10 and 100 μg/ml,
respectively (Fig. 1B, 1C). The magnitude of inhibition was
significantly increased when oocytes were pre-treated with
the extract (Fig. 1C). The 5-HT responses that were reduced
by the extract at high concentrations hardly recovered after
washout, in contrast to those reduced by lower concentrations (Fig. 1A, 1B).
**Effect of the extract on the 5-HT concentration-response curve**

To study the inhibitory mechanism of the extract on the 5-HT receptors, oocytes were treated with different concentrations of 5-HT in the absence and presence of the extract 1 μg/ml. The extract caused a shift of the 5-HT response curve to the right without a lower maximal response. The ED$_{50}$ of 5-HT was changed from 0.1 to 1 μM (Fig. 2).

**Inhibitory effect of S. venosa extract on NMDA and glycine receptors**

To examine the specificity of the inhibitory effect of the extract on 5-HT receptor, the effects of the extract on NMDA and glycine receptor functions in oocytes injected with cRNA of NR1a/NR2B cDNA or rat spinal cord RNA, respectively, were tested. The current response elicited by NMDA agonists (10 μM glutamate plus 10 μM glycine) was dose-dependently reduced by the extract with IC$_{50}$ of 200 μg/ml (Fig. 3A). The inhibitory effect of the extract easily disappeared after washout when compared to that of the noncompetitive NMDA receptor antagonist MK-801. The current response, of glycine receptor, elicited by 300 μM glycine was dose-dependently reduced by the extract with IC$_{50}$ of 500 μg/ml (Fig. 3B). Pretreatment of oocytes with the extract prior to application of either NMDA or glycine did not enhance the inhibitory effect of the extract (data not shown).

**Discussions and Conclusion**

The present study has demonstrated 5-HT tubers dose-dependently inhibited 5-HT-receptor. When the inhibition on dose-response curve of 5-HT was observed, the results suggested that the inhibitory effect is similar to that of a competitive antagonist. Moreover, the inhibitory effect of the extract on 5-HT receptor is rather specific compared to that on NMDA and glycine receptors.
Figure 3  Inhibitory effect of S. venosa extract on NMDA and glycine receptors. Oocytes injected with NR1a and NR2B cRNAs or rat spinal cord RNAs were applied with 10 μM glutamate plus 10 μM glycine (Glu-gly) or 300 μM glycine, respectively, in the absence (control) or presence of the extract at 0.1 – 1,000 μg/ml. A) Examples of NMDA currents inhibited by the extract at different concentrations (10 – 1,000 μg/ml). MK-801 (10 μM), a noncompetitive NMDA receptor antagonist, was tested as a positive control. B) Examples of glycine currents inhibited by the extract at different concentrations (1 – 1,000 μg/ml). C) Data are expressed as the mean ± S.E.M. of percentages of control response from 2 - 8 oocytes.

The water extract from S. venosa tubers is a crude extract. Many constituents in the extract might involve in the observed inhibitory effect on 5-HT receptor. Using gas chromatography (GC) and mass spectrometer (MS) suggested that domestine and tetrahydropalmatine (THP) are the main components of the water extract from S. venosa tubers. Biological activity of domestine was not clearly identified. In contrast, many evidences demonstrated that THP possesses various pharmacological activities such as analgesic, sedative, hypnotic, and antihypertensive. Animal experiments have shown that the sedative effect of THP results from blocking dopaminergic neurons in the brain, and the benzodiazepine mediates, at least in part, such the effect. Many reports showed that blocking serotonin activity involved the antihypertensive and antinociceptive actions of THP. Although our previous observation found that animals injected with water extract from s. venosa tubers showed sedative effect, there is no evidence to support the involvement of THP and 5-HT blocking in sedative action. Other constituent (s) rather than THP might involve 5-HT blocking in sedative action of the extract. It is interesting to further examine which compound (s) in the extract acts as the 5-HT receptor antagonist.

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References


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**Editorial note**

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