Possible anti-tumor promoting properties of a total of 40 methanol extracts from Thai edible plants, including 5 non-edible species, were estimated \textit{in vitro} by an inhibition test of tumor promoter-induced Epstein-Barr virus (EBV) activation. Fourteen species (35% of the total) were found to be strongly active. In addition, cardamonin (2',4'-dihydroxy-6'-methoxychalcone) was identified as a potently active constituent (IC\textsubscript{50} = 3.1 \mu M) of \textit{Boesenbergia pandurata} (Zingiberaceae), suggesting it to be an effective anti-tumor promoter.

Since the two-stage carcinogenesis (initiation-promotion) theory\textsuperscript{11} was generally accepted, the inhibition of tumor promotion (anti-tumor promotion) has been regarded as one of the most promising ways for cancer chemoprevention.\textsuperscript{2,3} We have been searching for naturally occurring anti-tumor promoters by using a short-term \textit{in vitro} assay, the Epstein-Barr virus (EBV) activation test.\textsuperscript{3-5}

Hitherto, we have reported the anti-tumor promoting properties of edible plants and marine algae which are commonly ingested in Japan\textsuperscript{6-8} and also some of their active constituents.\textsuperscript{8,9} In order to search for more potent anti-tumor promoters from daily food items, we have focused on the edible plants that possess some medicinal effects. For this purpose, the edible plants of Thailand were selected because they are rich in such plant species that are used for purposes other than nutritional value: flavors, condiments, and occasionally traditional medicines. We describe here the results of screening tests on their inhibitory activities against EBV activation. In addition, the isolation and identification of an active constituent, cardamonin, from \textit{Boesenbergia pandurata} as well as its activity are reported.

A total of 40 fresh edible plants, including 5 non-edible species, most of which are used as flavors or condiments, or are known to possess some medicinal effects, were extracted with methanol, and the extracts (20 \mu g/ml) were submitted to the inhibition test toward EBV activation induced by teleocidin B-4 (20 ng/ml) as previously reported.\textsuperscript{9} The inhibitory effect (IE) of each test extract toward EBV activation was classified as follows: ++ strongly active (IE \geq 70\%); + moderately active (70\% > IE \geq 50\%); ± weakly active (50\% > IE \geq 30\%); — inactive (30\% > IE). Additionally, the cell viability (CV) was also classified as follows: ++ highly toxic (30\% \geq CV); + moderately toxic (30\% > CV \geq 30\%); — non-toxic (CV > 70\%).

The results are shown in Table 1, in which 14 species were evaluated as “strongly active” (IE \geq 70\%) and 31 species were found to be “weakly active” or better (IE \geq 30). The proportions of strongly active plants (35\% of the total) and of the plants being more than “weakly active” (78\%) were about 4 and 3 times higher, respectively, in this study than those previously reported in the screening test of common edible plants in Japan.\textsuperscript{6} And yet, the experimental conditions in this study seemed to be more stricter than those used before, because the relative amounts of extracts to the tumor promoter were 5 times less in the present assay than in the previous test.\textsuperscript{6} It is, accordingly, indicated that our criteria for plant selection could greatly facilitate the search for potent anti-tumor promoters from edible plants. The screening data also demonstrate that most Zingiberaceae and Rutaceae plants possess strong inhibitory activity toward EBV activation, and concurrent marked cytotoxicity. We previously isolated a highly potent EBV activation inhibitor (IC\textsubscript{50} = 1.5 \mu M), 1'-acetoxychavicol acetate (ACA), from \textit{Languas galanga}.\textsuperscript{9} ACA exhibited high cytotoxicity at more than 25 \mu M against Raji cells (unpublished data). Hence, the plants inhibiting EBV activation with cytotoxicity against Raji cells were evaluated as being a group of very promising species for strong anti-tumor promoters.

As a first approach to search for the active constituents of these plants, we investigated \textit{Boesenbergia pandurata} (Zingiberaceae), called “krachai” in Thailand. The rhizome of this plant is used

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{structure_cardamonin.png}
\caption{Structure of Cardamonin and Its Inhibitory Effect on EBV Activation by Teleocidin B-4 (50 nM).}
\end{figure}

\footnote{To whom correspondence should be addressed.}
as a condiment and occasionally as a folk medicine for the treatment of colic, or as an aphrodisiac.

The fresh rhizome of *B. pandurate* (1 kg) was extracted with methanol, and the extract was partitioned between EtOAc and water. The EtOAc-soluble part was then purified by column chromatography on Wako gel C-100 (benzene/EtOAc), by two steps of prep. TLC (benzene/EtOAc and CHCl3/MeOH), and finally by prep. HPLC on μBondasphere C18 (MeCN/H2O, 1:1) to give an active compound (I, 75 mg). By a detailed comparison of its spectral data,

A compound I was identified as cardamonin (2',4'-dihydroxy-6-methoxychalcone, Fig.), which had previously been isolated from this plant.\(^{11}\)

Cardamonin exhibited cytotoxicity against Raji cells at more than 50 μM. However, it completely inhibited the EBV activation at a concentration of 25 μM with high cell viability (>90%) (Fig.). The activity of cardamonin (IC50 = 31 μM) was comparable with that of ACA (IC50 = 1.5 μM), and the compound is evaluated to be one of the most potent chalcone-type inhibitors against EBV activation thus far reported.\(^{12}\) Yamamoto *et al.* have recently reported that isoliquiritigenin (4,2',4'-trihydroxychalcone), which is closely related to cardamonin, showed potent *in vivo* anti-tumor promoting activity in mice skin.\(^{13}\) Cardamonin is, hence, expected to possess similar potent activity *in vivo.*

A study on the anti-tumor promoting activity of cardamonin in mice skin and the search for further active compounds in other edible plants from Thailand are now in progress.

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**References and Notes**


10) Compound 1 (cardamonin; 2,4'-dihydroxy-6'-methoxycoumarin); UV λ_{max} (EtOH) nm (ε): 349 (21000); IR ν_{max} (KBr) cm^{-1}: 3400–2900 (br.), 1620; ^1H-NMR (400 MHz, acetone-d_{6}) δ: 3.99 (3H, s), 6.01–6.02 (1H, m), 6.09–6.10 (1H, m), 7.43–7.48 (3H, m), 7.72–7.78 (3H, m), 8.01 (1H, dd, J = 15.6 and 5.2 Hz); EIMS (70eV, probe) m/z (%): 270 (71, [M]^{+}, C_{16}H_{14}O_{6}), 269 (51), 253 (9), 193 (100), 178 (6), 167 (43).

