**REVIEW ARTICLE**

# Possible anti-tumour promoting properties of traditional Thai food items and some of their active constituents

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From a view point of cancer chemoprevention, possible anti-tumour promoting properties of daily food items and some of their active constituents have been investigated by a convenient in-vitro assay, the Epstein–Barr virus (EBV) activation test. In a screening test for the inhibitory activity toward EBV activation by 40 methanol extracts from Thai edible plants used for flavours, condiments or folk medicines, more than three-quarters of the total were found to possess inhibitory activities. Significantly, the ratio of activity-exhibiting plants was about three times higher than that of Japanese common vegetables and fruits previously studied. The two plant families of Zingiberaceae and Rutaceae, in particular, were suggested to be promising sources for highly effective anti-tumour promoters. Hitherto, geranial (*Cymbopogon citratus*, Gramineae), cardamonin (*Boesenbergia pandurata*, Zingiberaceae), curcumin (*Zingiber cassumunar*, Zingiberaceae) and 1′-acetoxychavicol acetate (*Languas galanga*, Zingiberaceae) have been identified as the active constituents of strongly active plants in the tumour promoter-induced EBV activation test. They showed more potent inhibitory activities than the representative anti-tumour promoters such as β-carotene or quercetin. The high potential of the traditional food items of Thailand in the search for potent anti-tumour promoters is described in this article.

## Introduction

### *Cancer chemoprevention*

Primary cancer prevention has mainly two aspects in its methodology: (1) exclusion or avoidance of the environmental carcinogens or other chemical factors closely relating to carcinogenesis such as tumour promoters; and (2) the administration of inhibitory or suppressive agents against carcinogenesis. Because the number of critical agents in cancer is almost untold, continuous exposure to such agents should be inevitable in daily life. Therefore, it has been widely accepted that the latter aggressive approach, direct chemical inhibition or suppression, should be rather efficient for the control of cancer incidence. Particularly, food phytochemicals could be important for cancer prevention. In fact, a great number of epidemiological studies of the relationship between food and cancer, together with the research in the experimental animal models, have demonstrated that daily ingestion of some vegetables and fruits could undoubtedly contribute to cancer prevention[1–4].

Cancer chemoprevention is a concept defined as the prevention of cancer by the administration of natural or synthesized pure chemicals, or by daily foods enriched with cancer preventive components[5–8]. As an initial step to human intervention trials, the target populations for cancer chemoprevention are generally recognized as so-called high-risk segments such as: (1) individuals in contact with certain carcinogens occupationally; (2) survivors from primary cancer with high possibility of recurrence; (3) individuals with a genetic history of a high frequency of cancer incidence; (4) individuals with predicted premalignancy by diagnosis with biomarkers; and (5) certain others. Though still controversial, cancer preventive agents may also be applicable to healthy people in the near future. In any case, because an infallible remedy for cancer has not been established yet, cancer chemoprevention may become a means to reduce cancer incidence.

### *Multistage carcinogenesis*

One ought to have several strategies for cancer chemoprevention because chemical carcinogenesis has been recognized to generally have three stages: initiation, promotion, and progression[9–11]. Characteristics at each stage are shown in Table 1. Initiation is a process provoked by some carcinogens causing point mutation(s) of the H-*ras* gene of the cellular DNA[12], which alters a normal cell into a dormant tumour cell. Promotion is considered to be a successive process caused by some tumour promoters accelerating the proliferation of the initiated cells, and the repetitive attack of tumour promoters to the initiated cells results in the formation of visible, benign tumour cells. The classical two-stage carcinogenesis hypothesis of initiation and promotion, first established in mouse skin, has recently been found valid in diverse types of cancer such as kidney[13], breast[14], lung[15, 16], stomach[17–19], and liver[20–24] in a variety of experimental animal models with several types of tumour promoters. Protein kinase C (PKC), an enzyme activated by endogeneous diacylglycerol released by an activation of phospholipase C, is widely accepted as one of the major intracellular targets of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-type tumour

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Table 1. Inhibitory activity of the methanol extracts of Thai plants toward EBV activation.

| Plant | Family name | PT[*1] | PU[*2] | IE[*3] | CV[*4] |
|---|---|---|---|---|---|
| *Acanthus ebracteatus* | Acanthaceae | leaves | M | ++ | – |
| *Rhinacanthus nasutus* | Acanthaceae | stem | M | ++ | – |
| *Volvariella volvacea* | Amanitaceae | stem | F | + | + |
| *Capparis micracantha* | Capparidaceae | stem | F, M | – | – |
| *Crataeva religiosa* | Capparidaceae | leaves | F, M | – | – |
| *Carica papaya* | Caricaceae | fruits | F, M | + | – |
| *Coccinia indica* | Cucurbitaceae | leaves | F, M | – | – |
| *Trichosanthes anguina* | Cucurbitaceae | fruits | F | – | – |
| *Gelonium multiflorum* | Euphorbiaceae | stem | M | +++ | – |
| *Cymbopogon citratus* | Gramineae | leaves | F, M | +++ | – |
| *Mentha arvensis* | Labiatae | leaves | F, M | – | – |
| *Ocimum basilicum* | Labiatae | leaves | F, M | + | – |
| *Barringtonia acutangula* | Lecythidaceae | leaves | M | +++ | – |
| *Acacia insuavis* | Leguminosae | leaves | F | – | – |
| *Cassia siamea* | Leguminosae | leaves | F, M | – | – |
| *Leucaena glauca* | Leguminosae | leaves, stem | F | + | – |
| *Neptunia oleracea* | Leguminosae | leaves | F | +++ | – |
| *Smilax sp.* | Liliaceae | stem | M | + | – |
| *Hibiscus esculentus* | Malvaceae | fruits | F | – | – |
| *Azadirachta indica* | Meliaceae | fruits | F, M | ++ | – |
| *Tiliacora trianda* | Menispermaceae | leaves | F, M | ++ | – |
| *Moringa oleifera* | Moringaceae | fruits | F, M | ++ | – |
| | | leaves | F, M | +++ | – |
| *Musa sapientum* | Musaceae | flower | F | +++ | – |
| *Nelunbo nucifera* | Nymphaeaceae | rhizome | F, M | + | – |
| *Olax scandens* | Olacaceae | leaves | F | +++ | – |
| *Morinda citrifolia* | Rubiaceae | leaves | F, M | +++ | – |
| *Citrus aurantifolia* | Rutaceae | fruits | F, M | +++ | + |
| *C. hystrix* | Rutaceae | leaves | F, M | +++ | ++ |
| *Capsicum annuum* | Solanaceae | fruits | F, M | ++ | + |
| *C. frutescens* | Solanaceae | fruits | F, M | – | – |
| *Amomum krervanh* | Zingiberaceae | rhizome | F | + | – |
| *Boesenbergia pandurata* | Zingiberaceae | rhizome | F, M | +++ | ++ |
| *Curcuma mangga* | Zingiberaceae | rhizome | F, M | ++ | + |
| *Kaempferia galanga* | Zingiberaceae | rhizome | F, M | ++ | + |
| *Languas galanga* | Zingiberaceae | rhizome | F, M | +++ | ++ |
| *Nicolaia elatior* | Zingiberaceae | rhizome | F | + | – |
| | | leaves, stem | F | ++ | – |
| *Zingiber cassumunar* | Zingiberaceae | rhizome | F, M | +++ | + |
| *Z. zerumbet* | Zingiberaceae | rhizome | F, M | +++ | – |

*1 Part for use.
*2 Purpose of use: F, foodstuff; M, medicine.
*3 Inhibitory effect.
*4 Cytotoxicity.

promoters[25–27]. PKC activation is involved in the phosphorylation of proteins regulating cellular differentiation and/or proliferation. However, the detailed mechanisms of tumour promotion are not clear. Progression, a relatively new concept, is a process involving invasion of tumour cells into the surrounding tissues or metastasis to distant organs. Some substances acting as progressors or anti-progressors have been reported recently[28–30].

In order to prevent cancer, it would be effective to block these three stages independently or concurrently. We have been focusing on the inhibition of tumour promotion (anti-tumour promotion), because the promotion stage is known to need long term for completion and also to be the only process possessing reversibility.

*The Epstein–Barr virus (EBV) activation test*

To search for effective anti-tumour promoters, we have conducted a convenient *in-vitro* assay, the Epstein–Barr virus (EBV) activation test[31]. The EBV, classified as a herpes virus, is known to be distributed around the world[32]. Significantly, EBV has been considered to be associated with some human cancers such as African Burkitt's lymphoma or anaplastic nasopharyngeal carcinoma[33–37]. zur Hausen, and independently Ito, found that EBV latently infected the human B lymphoblastoid cell, Raji, was highly activated by some tumour promoters such as TPA or teleocidins[31, 38].

Thus, the inhibition of EBV activation might reflect anti-tumour promotion in animal models. In fact, most of the EBV activation inhibitors from several medicinal and Japanese edible plants, were proven to possess significant anti-tumour promoting activity in mouse skin in our laboratory[39–50].

*Thai vegetables and fruits*
Thailand is widely known to have a rich flora with the populace making frequent use of unique vegetables or fruits for flavours, spices or condiments in their traditional cuisine. Furthermore, there are a large number of edible plants which are concurrently used as traditional folk medicines. It should also be noted that Thailand still has many species of vegetables almost never given to plant breeders[51], giving rise to the possibility that they may contain some biologically active compounds not occurring in long-bred plants, or may surpass in the quantity of active phytochemicals. Thus, Thai edible plants, especially those eaten for purposes other than nutrition may be promising sources for biologically active compounds including anti-tumour promoters.

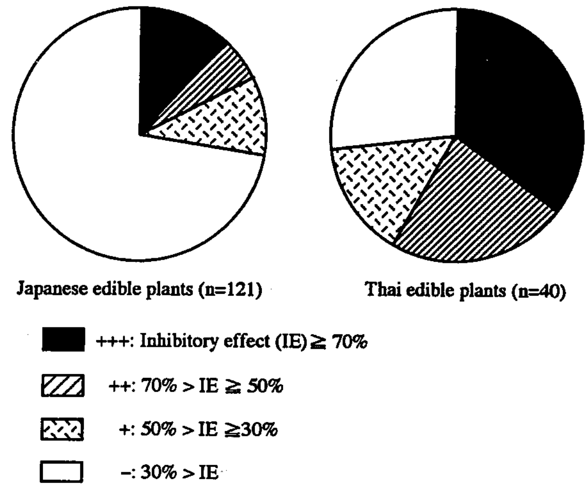



Figure 1. The proportions of the EBV activation inhibitory activities of the extracts from Thai and Japanese edible plants.

## Screening tests for the inhibitory activity toward Epstein–Barr virus (EBV) activation of Thai edible plant extracts

A total of 40 methanol extracts (20 µg/ml) from Thai vegetables and fruits, particularly those used as flavours or condiments, were submitted to the EBV activation test, in which teleocidin B-4 (20 ng/ml), a potent TPA-type tumour promoter[52], and sodium *n*-butyrate (3mM), were used as the EBV activator[48]. EBV genome activation was measured by the level of induction of viral early antigen (EA). Possible anti-tumour promoting activity was determined by the ratio of EA-induced cells treated with a test sample as compared with that tested with only *n*-butyrate and teleocidin B-4. The inhibitory effect (IE) of each test extract was classified as follows: +++ strongly active (IE≧70 %); ++ moderately active (70 %>IE≧50 %); + weakly active (50 %>EI≧30 %); – inactive (30 %>IE). In addition, cell viability (CV) was also classified as follows: ++ highly toxic (30 %≧CV); + moderately toxic 70 %≧CV>30 %; – non-toxic (CV>70 %).

Screening data (Table 2) shows that 31 species, consisting of 14 strongly, nine moderately, and eight weakly active species, exhibited significant inhibitory activity toward EBV activation. As shown in Figure 1, the proportion of strongly active plants (35 % of the total) to the whole was about three times higher than that (12 %) in the screening test of Japanese common edible plants tested previously[41], though there is an obvious difference in their sample numbers. The experimental conditions in the present test seem to be stricter than those in the previous screening test for Japanese common edible plants, since the relative concentrations of the extracts to the tumour promoter were five times less in the present assay than in the previous test[41]. It is, accordingly, indicated that our criteria in plant selection centring on flavours, condiments etc could greatly facilitate the search for natural sources of potent anti-tumour promoters. One might ask if there are notable differences in activity between the common nutritive Thai and Japanese plants. To clarify this, further screening tests of 150 common edible plants from Thailand are now being undertaken.

The screening data (Table 2) also indicate that the two plant families, Zingiberaceae and Rutaceae, are notable for their high frequency of remarkable inhibitory activities. We are mostly interested in three strongly active plant species, *Cymbopogon citratus*, *Citrus hystrix*, and *Languas galanga*, traditionally used as flavours and/or condiments for a famous shrimp soup in Thailand 'tom yam kung'.

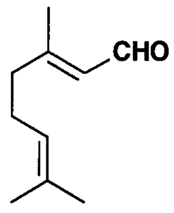
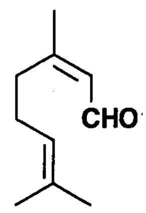
Table 2. Some characteristics of the stages in carcinogenesis.

| Stage | Characteristics |
|---|---|
| Initiation | Irreversible |
| | Requires fixation |
| | Additive |
| | No threshold |
| Promotion | Reversible |
| | Environmentally modulated |
| | Maximal response |
| | Threshold |
| Progression | Irreversible |
| | Somatic aneuploidy |
| | Progressive karyotypic instability |

Adapted from reference 11.

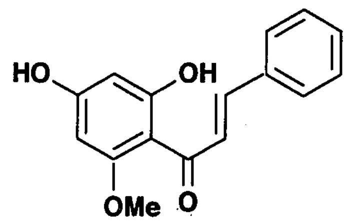
## Possible anti-tumour promoters from the Thai edible plants

*Cymbopogon citratus (lemon grass or lapine)*
*C. citratus* (Gramineae) is widely distributed throughout South-east Asia. The leaves and stem of the young plant are reported to have anti-fungal, repellent, and bacteriostatic activities[53]. It also has traditionally been used as a tonic or in perfume on account of its lemon-like fragrance. Two isomeric monoterpene aldehydes (Figure 2), geranial (*trans*-citral) and neral (*cis*-citral), were isolated as the EBV activation inhibitors from the leaves. As Connor has already reported the anti-tumour promoting activity of a mixture of these citrals in mouse skin[54], we independently examined each EBV activation inhibition activity of geranial and neral after being purified by HPLC (Novapak $C_{18}$, 50 % acetonitrile in $H_2O$).

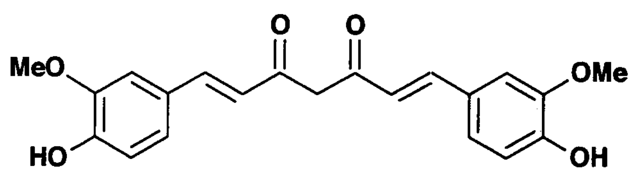
geranial neral

(*Cymbopogon citratus*)



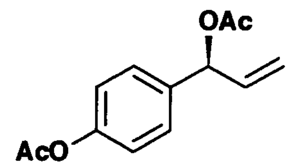
cardamonin

(*Boesenbergia pandurata*)



curcumin

(*Zingiber cassumunar*)



1'-acetoxychavicol acetate

(*Languas galanga*)

Figure 2. Possible anti-tumour promoters from Thai edible plants.

Table 3. Inhibitory activities of the constituents from the edible plants of Thailand toward EBV activation.

| Compound | Inhibitory activity ($IC_{50}$, μM) |
|---|---|
| Geranial | 16 |
| Neral | 130 |
| Cardamonin | 3.1 |
| Curcumin | 5.4 |
| 1´-acetoxychavicol acetate (ACA) | 1.5 |
| α-linolenic acid | 27 |
| (−)-epigallocatechin gallate | 68 |
| Quercetin | 23 |
| β-carotene | 30 |

The HPLC analysis also revealed that the content ratio of geranial and neral in *C. citratus* leaves was about three to two. As shown in Table 3 geranial was found to possess about eight times higher inhibitory activity than neral when compared by the 50% inhibition concentrations ($IC_{50}$). The mixture of geranial and neral from the leaves showed medium inhibitory activity between those of geranial and neral, indicating that the activity of citral is attributable to the additive effect of geranial and neral. However, it is still unknown whether the activity difference between them would be also observed in vivo. The $IC_{50}$ value of geranial (16 μM) in the EBV activation inhibition is comparable to that of β-carotene. As citral is already used as a food additive, its application to cancer chemopreventive foods might not meet any obstacles.

*Boesenbergia pandurata ('kra chaai' in Thai)*

*B. pandurata* (Zingiberaceae) is a cultivated herb in Thailand, and its rhizome has anti-fungal, carminative and vermifuge activities[53]. We identified cardamonin (Figure 2), 2´, 4´-dihydroxy-6´-methoxychalcone, as a potent EBV activation inhibitor from the plant[48]. The $IC_{50}$ value of cardamonin (3.1 μM) was remarkably lower than those of the known representative anti-tumour promoters (Table 3). Furthermore, cardamonin was evaluated to be one of the most potent chalcone-type inhibitors against EBV activation thus far reported[55]. Yamamoto et al have recently reported that isoliquiritigenin (4,2´4´-trihydroxychalcone), closely related to cardamonin, showed potent in vivo anti-tumour promoting activity in mouse skin[56]. Thus, cardamonin is expected to possess anti-tumour promoting activity comparable with isoliquiritigenin in vivo.

*Zingiber cassumunar ('plai' in Thai)*

*Z. cassumunar* (Zingiberaceae) is used as an embrocation or a carminative, and reported to possess anti-inflammatory effect[53]. We found a strong inhibitory activity toward EBV activation in a yellow-coloured fraction from a methanol extract of its rhizome. Curcumin (Figure 2), a dietary yellow pigment frequently used for some curries or mustard, was identified as one of the active constituents of the fraction[50]. Furthermore, a partially purified fraction without curcumin still showed potent inhibitory activity, indicating that the fraction might include further effective anti-tumour promoters. The isolation and identification of these components are now in progress. The $IC_{50}$ value of curcumin (5.4 μM) is evaluated to be moderate among the active constituents from Thai edible plants (Table 3). Nishino et al[57] and Huang et al[58] independently reported anti-tumour promoting effect of curcumin in mouse skin. As curcumin is known to be widely used as a food additive, there may be high potentiality for its application as a cancer preventive agent.

*Languas galanga (great galangal)*

*L. galanga* (Zingiberaceae) has a characteristic fragrance as well as pungency, and its rhizome is widely used as a condiment of soups or curries in Thailand. 1´-acetoxychavicol acetate (ACA, Figure 2), first reported as the anti-ulcer principle of the plant by Mitsui et al[59], was found to be the major inhibitor toward the EBV activation[49]. The $IC_{50}$ value of ACA (1.5 μM) is ten times lower than that of β-carotene, that is extensively studied in human intervention trial at the Chemoprevention Branch of the National Cancer Institute (NCI) of the USA[60]. The EBV activation inhibitory activity may very well be evaluated to be one of the most potent among the active compounds from edible sources. The quantity level of ACA in fresh rhizome was estimated to be 0.09 % by an HPLC analysis (Murakami et al, unpublished data). ACA showed a remarkably strong anti-tumour promoting activity in ICR mouse skin. The detailed in-vivo results as well as the structure–activity relationship study of ACA will be reported elsewhere.

## Conclusions

At the present time, several hundred chemical agents, consisting of at least 30 distinct chemical groups, have been reported as cancer preventive agents in experimental animal models[61], offering the possibility of application in cancer chemoprevention according to their efficacy. Furthermore, it is most noteworthy that over 30 clinical intervention trials are now underway at the NCI of the USA[8] as the result of widespread screening tests as well as the intensive studies on their activity, action mechanisms, and clinical safety. In other quarters as well, new types of cancer chemopreventive agents with high efficacy are much sought after, with the research on cancer chemoprevention at the clinical level having just been initiated recently. Certainly the search for cancer preventive agents from edible sources is also important. The potentiality of Thai edible plants as effective anti-tumour promoters seem to be high because cardamonin or ACA as pointed out in this study, exhibited higher inhibitory activity toward tumour promoter-induced EBV activation than the heretofore representative chemopreventive phytochemicals such as β-carotene or (–)-epigallocatechin gallate (Table 3). Needless to say, these EBV activation inhibitors from Thai plants must be further examined for their anti-tumour promoting activities in animal models. In addition to identification of further active constituents from strongly active Thai edible plants, investigation of the action and metabolism mechanisms, oral activity, and the assessment of clinical safety, are also required. Furthermore, combination tests of several types of anti-tumour promoters in a search for synergism might result in the design of highly effective cancer chemopreventive foods.

*Acknowledgements* – We would like to thank Professor Mark L. Wahlqvist for giving us an opportunity to write this review article.

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**Possible anti-tumour promoting properties of traditional Thai food items and some of their active constitutents**

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*Asian Pacific Journal of Clinical Nutrition 1994; 3: 185–191*

## タイ国伝統的食品素材の*in vitro*発癌プロモーション抑制作用と活性物質

### 要　　旨

タイ国において、料理の香り付けなどに用いられ、同時に薬用利用も知られる独特な野菜類に着目し、発癌予防作用について評価した。*in vitro*発癌プロモーション抑制活性の検定系であるEpstein-Barrウイルス活性化抑制試験を用いて、４０種のメタノール抽出物について試験したところ、全体の4分の３以上に顕著な抑制活性が認められた。興味深いことに、この割合は、日本において常食されている野菜や果実類についての試験で示された割合より約3倍高いものであった。なかでも、ショウガ科およびミカン科植物が高い活性を示す植物群であることが判明した。現在までに、ゲラニアール(*Cymbopogon citratus*、イネ科)、カルダモニン(*Boesenbergia pandurata*、ショウガ科)、クルクミン(*Zingiber cassumunar*、ショウガ科)、1'-アセトキシチャビコールアセテート(*Languas galanga*、ショウガ科)が活性物質として単離同定できた。これらはいずれも、Epstein-Barrウイルス活性化抑制試験において、代表的な既知活性物質である、β-カロチンやケルセチンなどよりも有意に高い活性を示した。以上の結果から、タイ国産の野菜類は、強力な発癌予防物質を得るうえで非常に有用であることが強く示唆された。

## 傳統泰國食物及其活性成份的可能抗腫瘤特性

### 摘要

作者從化學防癌的觀點，在體外用 Epstein-Barr 病毒 (EBV) 激活試驗，研究了每日食物及其活性成份的可能抗腫瘤特性。從用作調味品或民間傳統醫療的泰國食用植物中，獲得 40 種甲醇抽提物，用這些抽提物作抑制活性的篩選試驗，發現 3/4 以上具有抑制作用。明顯地，其活性一抑制植物的比例約高於以前研究過的，日本常用蔬菜和水果的 4 倍。特別是Zingiberaceae)和 Rutaceae 兩個植物家族，被認爲是高效抗腫瘤促進劑的良好來源。到目前爲止，老鶴草屬植物 (Cymbopogon citratus，Gramineae) 、小豆蔻屬植物 ( Boesenbergia pandurata，Zingiberaceae)、姜黄屬植物 (Zingiber Cassumunar，Zingiberaceae) 和 1'-acetoxychavicol acetate ( Languas galanga，Zingiberaceae) 已被鑒定含活性成份，是強烈的抗腫瘤促進劑，會引起 EBV 激活試驗。他們比較抗腫瘤促進劑的代表，如β一胡蘿白素或五羥黄酮 (Quercetin) 具更有效的抑制作用。該文描述了泰國傳統食物中含有效抗腫瘤促進劑的高度可能性。