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# Antimicrobial and antioxidation effects of Thai seasoning, *Tom-Yum*

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**Abstract**

*Tom-Yum*, a Thai traditional seasoning, as a potential functional food and a natural antimicrobial agent was investigated. Among the ingredients in *Tom-Yum*, garlic exhibited the highest antimicrobial effect on *Pseudomonas fluorescens* ATCC 49839, *Escherichia coli* O157:H 7, *Staphylococcus aureus* ATCC 13565 and *Listeria monocytogenes* with inhibition zones of 2.0 cm, while red chili and kaffir leaves were main sources of $\beta$-carotene at levels of $192.04 \pm 139.12\,\mu g/g$ sample and $173.60 \pm 61.45\,\mu g/g$ sample, respectively. However, no diazein or genistein was detected in any of the ingredients when LC-ESI-MS was used for confirmation.


*Keywords:* *Tom-Yum*; Antimicrobial; Disk diffusion method; Antioxidant; Pathogenic bacteria

## 1. Introduction

Thai food has gained popularity, and *Tom-Yum* is an important ethnic food consumed world wide due to its tastes, colors and health effect. In Japan and Thailand, researchers have discovered that some components found in galagal root, lemon grass and kaffir lime leaves, which are major ingredients of the soup, are effective in inhibiting tumors in the digestive tract (Division of Health Statistics, 1989; Murakami, Kondo, Nakamura, Ohigashi, & Koshimizu, 1993; Murakami, Ohigashi, & Koshimizu, 1994; Murakami, Kondo, Nakamura, Ohigashi, & Koshimizu, 1995). In addition, other ingredients of *Tom-Yum* include chili, shallot (red onion), and garlic which are natural antimicrobial, antioxidant compounds with health benefits (Nishimura, Takahashi, Wijaya, Satoh, & Ariga, 2000). Garlic and onion have generally found to be a great antibacterial (Shelef, 1983), antidiabetic, hypocholesterolemic and cancer preventive agent (Nishimura et al., 2000). Allicin, one of the active components of freshly crushed garlic homogenates, has a variety of antimicrobial, antifungal, antiparasitic, and antiviral activities. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (Ankri & Mirelman, 1999). Carotenoids are one of the most abundant groups of natural pigments because most living plants synthesize them as a protector against photo-oxidative processes and they are constituents of chromoplasts (Deli, Matus, Molnar, & Toth, 2001). However, content of carotenoids in vegetables greatly varies in amount, depending on spices, variety, time of the year, and degree of ripeness (Deli et al., 2001). Essential oil of lemon grass is mainly comprised of citral which exhibited a broad antifungal spectrum (Adegoke & Odesola, 1996; Schaneberg & Khan, 2002) while galangal root and kaffir lime leaves extracts failed to inhibit *Bacillus cereus*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus ochraceous*, or *Cryptococcus neoformans* (Mackeen et al., 1997). As researchers and consumers have increasing concern about potential health problems associated with synthetic additives, they are continuously focusing on the use of plant

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products as alternatives to synthetic ones. However, no scientific studies using whole *Tom-Yum* mix instead of individual active component as a functional food and natural preservative is available. The objective of this study was to determine the antibacterial effect and antioxidant content of the *Tom-Yum* mix and its ingredients.

## 2. Materials and methods

### 2.1. Antibacterial activity

*Bacterial culture*: Two Gram-negative bacteria: *Pseudomonas flurorescens* ATCC 49839, *E. coli* O157:H7 204P and two Gram-positive bacteria: *Staphylococcus aureus* ATCC 13565 and *Listeria monocytogenes* were used for target bacteria. *P. flurorescens* ATCC 49839 was obtained from the Department of Poultry Science while the others were provided by the Department of Food Science and Technology, The University of Georgia, Athens, GA, USA. Individual test bacteria were maintained on Microbank™ cryogenic beads (Pro-Lab Diagonstics, Austin, TX, USA) and aseptically subcultured on tryptone soy agar (TSA; Difco Labs, Division of Becton Dickinson and Co., Sparks, MD, USA), tryptone soy broth (TSB; Difco Labs) and brian heart infusion broth (BHI; Difco Labs) three times before use in experimentation. Five loopfuls of each of the test bacteria from BHI were aseptically transferred into 5 ml of BHI and incubated at 32 °C for 18 h before use. These culture conditions yielded approximately $10^9$ cfu/ml for each culture except *P. flurorescens* which yielded approximately $10^8$cfu/ml. Bacteria were plated on Plate Count Agar (PCA; Difco Labs); and TSA for 18 h at 32 °C which yielded a range of $10^5$–$10^9$ cfu/ml for disk diffusion testing (National Committee for Clinical Laboratory Standard, 2000).

*Spices material and preparation*: Fresh spices including garlic, shallot, kaffir lime leaves, chili, glangal, and lemon grass were purchased from an Asian farmer's market located in Atlanta, GA, USA. Garlic and shallot were stored at 5 °C and the other ingredients were frozen at −20 °C until used. Individual spices were washed with running tap water for 2 min and rinsed with sterilized distilled water in a ratio of spice to water of 1:2. The spices were then trimmed and cut into small pieces and ground in a mortar. All equipment has been cleaned and sanitized with 50 μg/g chlorine solution. Sterilized disks were impregnated in each ground spice for at least 12 h at 10 °C and then dried in a biological hood for 15 min to remove excess liquid before it was put on PCA and TSA plates inoculated with the bacteria mentioned above. The plates were incubated at 32 °C for 18 h to determine inhibition zones.

*β-carotene content*: The method of β-carotene analysis was slightly modified from Limroongreungrat and Huang (2002) by using HPLC technique. Trans β-carotene was purchased from Fluka Bio Chemika (St. Louis, MO, USA). Stock solutions of β-carotene were prepared by placing 5 mg of trans β-carotene into a 25 ml volumetric flask and adjusting the volume with hexane. Absorbance difference was determined at 453 nm and the concentration of stock solution was calculated from the coefficient of β-carotene ($E^{1\%}1\,cm = 2592$). Six concentrations of standard solution (0.5–4.0 μg/ml) were used to plot the standard curve.

*Liquid chromatographic system and conditions*: The chromatograph consisted of C18 SUPLEX PKB-100 column (25 cm × 4.6 mm, 5 μm, 120 Å) (Supelco, USA), a Thermo Separation pump (Thermo Finigan, San Jose, CA, USA), and Isoco V4 absorbance detector (Isoco Inc., Lincoln, NA, USA) set at 450 nm. The isocratic separation was performed and the flow rate of the mobile phase was adjusted to 1 ml/min. The peak area was determined by using ChromJet integrator Model SP4400 (Thermo Separation Products). Two 10 g portions (depending on the strong yellow or red color) of the edible part of the tested samples were ground with isopropanol and hexane containing 0.1 g/100 ml BHT three times in the ratio of 15:25, 10:15 and 10:15 (ml:ml) by using a homogenizer (Omni Mixer Homogenizer, Waterbury, CT, USA) for 2, 1 and 1 min, respectively. For removing water from the sample, 5–7 g of magnesium sulfate was added during the first homogenization. The homogenate sample was vacuum filtered, and the filtrate was transferred to a 100 ml volumetric flask. The volume was adjusted with hexane containing 0.1 g/100 ml BHT. One 3-ml portion of each sample was dried with $N_2$ and dissolved in 1 ml of mobile phase, actonitrile/methanol/tetrahydrofuran (25/28/2, ml/ml/ml).

*Isoflavone determination*: Isoflavone determination was modified from Hutabarat, Mulholland, and Greenfield (1998) by using HPLC techniques and confirming the results by using LC-ESI-MS techniques operated by the Department of Chemistry at The University of Georgia, Athens, GA, USA. The edible part of the test sample was chopped and ground with a Handy Chopper (Black and Decker™ Inc., Shelton, CT, USA). One 3-g portion was mixed with 10 ml of HCl and 40 ml of EtOH containing 0.05 g/100 ml BHT in a boiling flat bottom flask and then sonicated for 25 min before refluxing at 80 °C for 9 h. The extracted sample was cooled down at room temperature and then vacuum filtered. The filtrate was transferred to a 50 ml volumetric flask and volume was adjusted with EtOH containing 0.05 g/100 ml BHT. One 3-ml portion of each sample was dried with $N_2$ and dissolved in 1 ml of mobile phase, acetonitrile/water+acetic acid (99/1) (33/67, ml/ml).

Table 1
Bacterial load in various samples

| Sample | Bacteria count[a] |
|---|---|
| Garlic | $<10^2$ cfu/g |
| Shallot | $<10^2$ cfu/g |
| Kaffir lime leaves | $10^4$–$10^5$ cfu/g |
| Red chili | $10^3$–$10^4$ cfu/g |
| Green chili | $10^3$–$10^4$ cfu/g |
| Galangal (young part) | $10^4$–$10^6$ cfu/g |
| Galangal (old part) | $10^2$–$10^4$ cfu/g |
| Lemon grass | $10^4$–$10^7$ cfu/g |
| Fresh *Tom-Yum* mix | $10^2$–$10^4$ cfu/g |
| Fresh *Tom-Yum* mix (juice) | 10–$10^2$ cfu/ml |
| Commercial *Tom-Yum* product | |
| 3.5 g/100 g citric acid | ND |
| 4.5 g/100 g citric acid | ND |
| 5.5 g/100 g citric acid | ND |
| | ND |

[a]Triplicate plates were tested per sample per experiment and each sample was run from three different lots.

*Statistical analysis*: The experiment was repeated three times and was conducted on separate lots of materials on different weeks. The study was designed as a randomized complete block design and the separate lot served as the blocking variable. Mean comparisons were performed using HSD the Tukey test to examine differences between treatments. All analyses were performed with SPSS statistical software package.

## 3. Results and discussion

Bacterial loads of the different samples are presented in Table 1. The results show that lemon grass and galangal root were heavy contaminated with bacteria, while garlic and shallot had low bacterial loads. Spices and natural agriculture seasoning materials are commonly contaminated with microorganism including bacteria, mold and yeasts (Alemela, Nieto-Snadoval, & Lopez, 2002). However, the number and type of microorganisms may vary with material, origin, climatic conditions, harvesting, storage and transport methods used, packaging and general environmental and handling circumstances, including the nature and extension of quality control measure. Although spices used in the formulation of *Tom-Yum* mix may be contaminated with microorganisms, when they were used for formulation as *Tom-Yum* mix, bacterial population reductions due to garlic was exhibited and will be described later.

Because the results obtained from PCA and TSA plates are the same in terms of the amount of viable counts and dimensions of clear zone, PCA was used for the experimentations. In addition, bacterial loads in the range of $10^5$–$10^7$ cfu/ml were suitable to determine the disk diffusion test. Antibacterial activities of test samples are present in Table 2. The results indicate that garlic has the strongest effective inhibition among *Tom-Yum* ingredients. Some researchers believe allicin (diallyl thiosulfinate) is the principle antimicrobial compound of fresh crushed garlic (Ankri & Mirelman, 1999; Miron et al., 2000);however, when the garlic was heated, there had no inhibition zones around the test bacteria. The possibility that this compound is not heat stable or may convert to some form that has no or little antimicrobial activity which agrees with the results of Wilknison (1997) and Ankri and Mirelman (1999). Moreover, adding water (approx. 3 g/100 g) did not reduce antibacterial property but adding edible oil (approx. 3 g/100 g) reduced this property. This may be due to miscible of the active component and oil interfering with the hydrolysis system. There was no antibacterial activity from shallot even though it is in the same family with garlic. Ankri and Mirelman (1999) detected no allin, the precursor for allicin, in onion while Xiao and Parkin (2002) found isoalliin and cyloalliin in onion instead of alliin. Regardless, the *Tom-Yum* mix and kaffir lime leaves have potential antibacterial effects even though some scientific experiments failed to find the antibacterial property by using the disk diffusion method (Mackeen et al., 1997). There was no antibacterial effect in lemon grass, galangal root or chili in this study. Some experiments found that capsaicin, the main active compound for pungency or heat sensation, has an antibacterial property against *Helicobacter pylori* (Jones, Shabib, & Sherman, 1997). However, this compound has low solubility in water (Santamaria, Reyes-Duarte, Barzana, Fernando, Mota, & Lopez-Munguia, 2000) and that may be the reason why chili had no effect on any test bacteria. Moreover, it is possible that the concentration and/or purity of active compounds in each spice are not enough to inhibit test bacteria. Since antimicrobial properties depends on several factors such as type, composition and concentration of the spices, and concentration of target microorganisms (Fung, Lin, & Gailani, 1985). Other factors include the extraction methods as well as forms used (i.e. essential oil form Fung et al., 1985) or crude extract which was used in this experiment.However, when these ingredients combine to make *Tom-Yum* mix, the mix shows antibacterial activities. Garlic, in certain amounts, still has enough potential to express its antibacterial property so it could be increased in the formulation making *Tom-Yum* mix more antibacterial assuming consumer acceptance. Citric acid, which is normally used for lime juice substitution in commercial product, also expressed its antimicrobial activity when the concentration was higher due to lowering the pH. This is an alternative choice to consider for a new lot formulation. Genetics of the individual test bacteria may also play a role.

Table 2
Antibacterial activities on test bacteria from various samples

| Sample | Test bacteria | | | |
|---|---|---|---|---|
| | *E. coli* O157: H7 | *P. flourescens* | *S. aureus* | *L. monocytogens* |
| *Tom-Yum* mix (fresh) | ++ | – | ++ | ++ |
| Turmaric | – | – | – | – |
| Shallot | – | – | – | – |
| Kaffer lime leaves | – | – | + | – |
| Chili | | | | |
| Red chili | – | – | – | – |
| Green chili | – | – | – | – |
| Seed | – | – | – | – |
| Galangal | – | – | – | – |
| Lemon grass | – | – | – | – |
| Garlic | | | | |
| Whole garlic | +++ | +++ | +++ | +++ |
| Garlic heart | – | –/+ | – | – |
| Cooked whole garlic | – | – | – | – |
| Garlic + Galangal | ++ | ++ | ++ | ++ |
| Garlic + water | +++ | ++ | +++ | +++ |
| Garlic + edible oil | + | + | + | + |
| Edible oil | – | – | – | – |
| 3.5 g/100 g citric acid | –(0) | –(0) | –(0) | –(0) |
| 4.5 g/100 g citric acid | –(0) | + | + | –(0) |
| 5.5 g/100 g citric acid | –(0) | ++ | ++ | ++ |

–No inhibition or clear zone.
–(0) clouded inhibition zone.
+ Small clear zone (dimension <1.5 cm).
++ Medium clear zone (dimension 2.0< or >1.5 cm).
+++ Large clear zone (dimension >2 cm).

Table 3 shows that chilies in the red stage were a main source of β-carotene. The finger chili pulp had the highest β-carotene levels ($p<0.05$). Whole finger chilies have β-carotene levels lower than the pulp, which implies that the seed is not the source of this component. This agrees with HPLC area peak (data not showed) where the peak area was very small. Beside the red Thai chili used in the fresh *Tom-Yum* formulation, kaffir lime leaves, particularly medium or old stage, could be a second major source of β-carotene in the mix. Mature leaves contained more β-carotene than young leaves and this supports the findings of Deli et al. (2001) and Hornero-Mendez, de Guevara, and Minguez-Mosquera (2000) but contrasted with results of Deli, Matus, and Toth (1996) and Deli and Toth (1997). The amount of β-carotene in chili from this study seemed higher than previous reports which are in the range of 1.80–159.00 μg/g sample (Bhaskarachary, Roa, Deoshale, & Reddy, 1995; Howard, Talcott, Brenes, & Villalon, 2000; Hussein et al., 2000). There are several variables that can affect β-carotene content. Such examples are species, variety, time of year and ripeness (Deli et al., 2001) and even extraction method including the solvent and saponification step. Some researchers address that the saponification step yields higher β-carotene content (Minguez-Mosquera & Hornero-Mendez, 1994; Hart & Scott, 1995; Howard et al., 2000), while several papers recently state that the saponification step causes a lower yield of this compound (Oliver, Palou, & Pons, 1998; Granado, Olmedilla, Gil-Martinez, & Blanco, 2001). No isoflavone in any test samples was found even though the retention time of those was identical to standard daizein and genistein found in legume plants.

## 4. Conclusion

Fresh garlic had the highest antimicrobial property of the spices examined in this experiment. Garlic inhibited all test bacteria, followed by *Tom-Yum* mix and kaffir lime leaves whereas shallot, lemon grass, galangal and chili had no effect. This study indicated that red chili and kaffir lime leaves were the major source of β-carotene in *Tom-Yum* mix. *Tom-Yum* has potential as

Table 3
Summary of β-carotene content in test samples[1]

| Sample | β-carotene content μg/g sample[2] |
|---|---|
| Shallot | 0.15 ± 0.32 f |
| Garlic (whole) | 0.20 ± 0.42 f |
| Garlic (flesh) | 0.00 ± 0.00 f |
| Garlic (green heart) | 2.51 ± 0.84 f |
| Lemon grass (whole) | 2.19 ± 3.67 f |
| Lemon grass (wooden)* | 3.10 ± 1.54 f |
| Lemon grass (biggest)** | 0.00 ± 0.00 f |
| Lemon grass (leafy)*** | 0.37 ± 0.34 f |
| Galangal (whole) | 0.89 ± 0.25 f |
| Galangal (bark) | 0.69 ± 1.02 f |
| Galangal (xylem) | 0.00 ± 0.00 f |
| Galangal (dried) | 0.00 ± 0.00 f |
| Kaffir lime leaves (medium) | 173.60 ± 61.45c |
| Kaffir lime leaves (young) | 78.80 ± 34.06 d |
| Thai chili (green) | 12.85 ± 8.17 ef |
| Thai chili (orange) | 30.63 ± 9.19 e |
| Thai chili (red) | 192.04 ±139.12 b |
| Finger chili (whole) | 204.75 ± 46.72 b |
| Finger chili (pulp) | 287.19 ± 36.35 a |
| *Tom-Yum* (fresh) | 2.96 ± 0.72 f |
| Commercial *Tom-Yum* A | 5.09 ± 1.22f |
| Commercial *Tom-Yum* B | 7.68 ± 1.40 f |

[1]Based on fresh weight.
[2]Each value represents the mean and standard deviation from three lots. a–f means within a row with a different letter are significantly different ($P<0.05$).
*Plant part that above the ground BY about 1 cm which is wood and light yellow.
**Plant part that above the ground about 2–3 cm which has biggest dimension.
***Plant part that above the ground about 8–10 cm which is green leafy.

a natural preservative agent for ensuring safe marinated food products.

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