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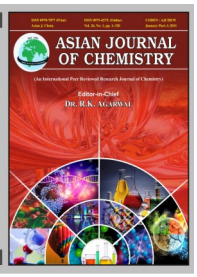

# Comparative Bioactivities Study of Five Dietary Plants Used in Malaysia

F.C. WONG[1,2,*], Y.V. YAP[2] and T.T. CHAI[1,2]

[1]Centre for Biodiversity Research, Universiti Tunku Abdul Rahman, 31900 Kampar, Malaysia
[2]Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Malaysia

*Corresponding author: Fax: +60 5 4661676; Tel: +60 5 4688888, Ext: 4521; E-mail: wongfc@utar.edu.my

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The purpose of this study was to determine the bioactivities and phytochemical contents of five food plants used in Malaysia. Radical scavenging activities were determined based on measurements of DPPH at 517 nm. Total phenolic and hydroxycinnamic acid contents were determined using spectrophotometric methods. Antibacterial activities were measured using Kirby-Bauer susceptibility test. Our results suggested that *S. torvum* is an ideal source of radical scavengers and total phenolics, while *G. atroviridis* could potentially be exploited to yield broad-range antibacterial therapeutic agents.

**Keywords: Antibacterial, Antioxidant, Caffeic acid, Hydroxycinnamic acid, Phenolic acid, Radical scavenging activities.**

## INTRODUCTION

Members from Plantae kingdom contributes to a significant portion of human diets in all continents. The parts consumed include but not limited to leaves, seeds, tubers, flowers and fruits. These food plants are consumed and incorporated into our culinary in a variety of imaginable ways. With their richness in dietary fibers, minerals, proteins and vitamins, dietary plants contribute to the basic metabolic needs of human bodies. Additionally, various secondary metabolites unique to food plants are necessary to maintain our overall well-beings[1]. These diverse groups of plants' secondary metabolites, such as phenolic acids, flavonoids and tannins, are frequently good sources of radical scavengers[2], which are essential to neutralize or eliminate the reactive oxygen species generated in normal cellular metabolism or resultant from pollutions[3].

Studies have linked the direct relationship between diets rich in plant-derived phytochemicals to a decreased occurrences of various cancer or malignancy[4]. In addition, many plant-derived phytochemicals have also been reported to be excellent antimicrobial agents. Many of these plants have further been exploited commercially to yield therapeutic drugs used in modern medicine. Well-known examples include vinblastine and vincristine, two alkaloid drugs widely used in chemotherapy, which are derived originally from *Catharanthus roseus* (Madagascar periwinkle)[5]. Moreover, camptothecin analogs such as anti-cancer irinotecan and topotecan, were originally derived from *Camptotheca acuminata* (Asian happy tree)[6,7]. Likewise, antimicrobial farnesyl hydroquinones (Ganomycin A and B) isolated from *Ganoderma pfeifferi*, a medicinal mushroom species, were found to inhibit the growth of methicillin-resistant *Staphylococcus aureus*[8].

Although many food plants are used widely and their benefits acclaimed, more studies are needed to study and document their benefits in a scientific, quantitative manner. In this study, we aim to measure and compared the bioactivities of five selected dietary plants, namely *Archidendrum jiringa*, *Castanea sativa*, *Garcinia atroviridis*, *Solanum melongena* and *Solanum torvum*. These plants are consumed widely in Malaysia and other parts of Southeast Asia. *A. jiringa* is acclaimed to be beneficial for diabetes patients[9]. *C. sativa* is popular as a healthy food, as it is rich in dietary fiber and minerals, but low in calories. *G. atroviridis*, known locally as 'asam gelugur', is consumed as salad mixture. *G. atroviridis* is believed to enhance blood circulation and reduce cholesterol level[10]. It is also used in traditional medicine to treat irritation and cough relief. *S. melongena* and *S. torvum* represent two varieties of eggplants widely consumed by the locals in their diets. Acclaimed functional benefits include treatment of fever, wound healing, arthritis relief and arterial hypertension[11].

## EXPERIMENTAL

**Preparation of plant extracts:** Food plant samples were purchased from local food stores in December 2011 and January 2012. The samples were incubated in an oven at 40 ºC for 48 h or until constant weight was observed. Each dried plant samples was then macerated for 24 h in 90 % ethanol [1:10 (w/v)] at room temperature. Ethanol extracts were

combined and filtered. Filtered extract was then concentrated and dried under reduced pressure. Crude extract was stored at -20 ºC until testing.

**Determination of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity:** DPPH radical scavenging activity was assessed as described previously with modifications[12]. After 50 µl of extract was added, the DPPH mixture was left in the dark for 0.5 h before its absorbance was read at 517 nm. DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ = absorbance of control reaction (without plant extract) and $A_{sample}$ = absorbance in the presence of a plant extract. A blank was prepared for each sample in which the DPPH solution was replaced with water.

**Determination of total phenolic content:** The concentrations of total phenolic in the plant extracts were determined using a Folin-Ciocalteu colorimetric assay, as described previously with modifications[13]. A mixture of extract (0.1 mL) and 10 % (v/v) Folin-Ciocalteu reagent (0.2 mL) was first incubated at room temperature for 3 min. After the addition of 0.8 mL of 700 mM $Na_2CO_3$ followed by incubation for 2 h, the absorbance of the mixture was read at 765 nm. A standard curve was prepared from gallic acid. Total phenolic content was expressed in mg gallic acid equivalents/g dry matter.

**Determination of hydroxycinnamic acid content:** The hydroxycinnamic acid content was determined as describe previously[14]. Briefly, 1 mL of plant extract was mixed with 2 mL of 0.5 M hydrochloric acid, followed by the addition of Arnow reagent and NaOH. After dilution to 10 mL, the absorbance was recorded at 490 nm. The total hydroxycinnamic acid contents were determined using a caffeic acid standard curve.

**Kirby-Bauer antimicrobial susceptibility test:** The antibacterial activities of the selected plant extracts were tested using Kirby-Bauer disk diffusion method, against both Gram-positive (*Staphylococcus aureus* and *Micrococcus luteus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains. Briefly, bacterial culture inoculum was adjusted to 0.5 McFarland standard before swapped onto the MHA agar. Sterile forceps were used to apply filter paper disks containing plant extracts onto the agar surface. Tetracycline, chloramphenicol and ampicillin antibiotic disks (Oxoid Ltd.) were used as positive controls. MHA plates were incubated for 18-24 hours at 37 ºC and diameter of the inhibition zone was measured (in mm).

## RESULTS AND DISCUSSION

**DPPH radical scavenging activity:** The plant extracts were tested in a DPPH assay to determine their free radical scavenging activities. All samples exhibited DPPH radical scavenging activities in a concentration-dependent manner, in a range from 20 to 80 mg/mL (Fig. 1). Compared at the concentration of 20 mg/mL, *S. torvum* (54%) possessed the highest radical scavenging activity, followed by *C. sativa* (50 %), *S. melongena* (43 %) and *G. atroviridis* (24 %), while *A. jiringa* (15 %) exhibited the lowest radical scavenging activity.

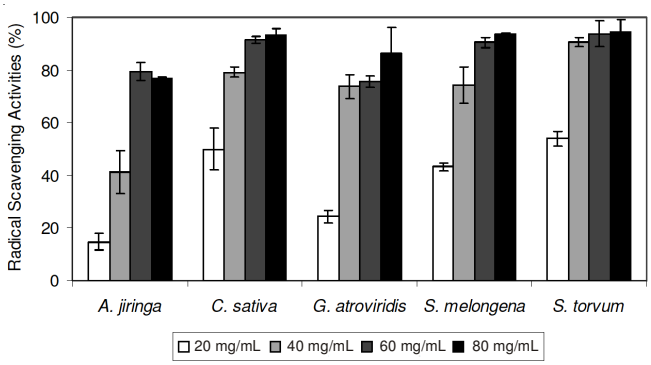


Fig. 1. DPPH radical scavenging activities of plant extracts at different concentrations. Data are reported as mean ± SE values (n = 3)

**Antibacterial Activities:** Plant extracts were also tested for their antibacterial activities against both Gram-positive (*S. aureus* and *M. luteus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains, using Kirby-Bauer disk-diffusion method (Table-1). *G. atroviridis* exhibited moderate (> 10 mm) to strong (> 20 mm) inhibition activities against all bacterial strains tested. Its inhibition activities against *M. luteus* and *P. aeruginosa* were comparable to that observed with commercial tetracycline. Both *A. jiringa* and *S. melongena* exhibit moderate inhibition activities against *M. luteus*. Weak (< 10 mm) or no antibacterial activity was detected for both *C. sativa* and *S. torvum*.

**Total phenolic and hydroxycinnamic acid contents:** As the bioactivities of plant extracts were often linked to their

TABLE-1
ANTIBACTERIAL ACTIVITIES OF PLANT EXTRACTS

| Plants | Zone of Inhibition (in mm) | | | |
|---|---|---|---|---|
| | *S. aureus* | *M. luteus* | *E. coli* | *P. aeruginosa* |
| *A. jiringa* | 0 | 11 ± 1[(b)] | 0 | 0 |
| *C. sativa* | 0 | 0 | 0 | 0 |
| *G. atroviridis* | 24 ± 1 | 41 ± 2 | 15 ± 1 | 22 ± 1 |
| *S. melongena* | 0 | 11 ± 2 | 0 | 9 ± 1 |
| *S. torvum* | 7 ± 2 | 0 | 8 ± 1 | 0 |
| Tetracycline[(a)] | 36 ± 1 | 49 ± 1 | 27 ± 3 | 19 ± 1 |
| Chloramphenicol | 31 ± 1 | 51 ± 1 | 11 ± 1 | 0 |
| Ampicillin | 49 ± 2 | 60 ± 3 | 9 ± 1 | 0 |

[(a)]Commercial tetracycline (30 µg), chloramphenicol (30 µg) and ampicillin (10 µg) disks (Oxoid Ltd.) were included as positive control.
[(b)]Values reported as mean ± SE values (n = 3)

TABLE-2
TOTAL PHENOLIC AND HYDROXYCINNAMIC ACID CONTENTS OF THE FIVE PLANT EXTRACTS

| Plants | Total phenolics (mg GAE/100 g dry matter) [a] | Hydroxycinnamic acids (mg CAE/100 g dry matter) [b] |
|---|---|---|
| *A. jiringa* | 356.2 ± 4.2[c] | 13.2 ± 0.2 |
| *C. sativa* | 216.3 ± 10.1 | 4.8 ± 0.1 |
| *G. atroviridis* | 455.6 ± 3.0 | 5.9 ± 0.4 |
| *S. melongena* | 554.2 ± 2.1 | 26.3 ± 2.4 |
| *S. torvum* | 672.4 ± 3.2 | 39.5 ± 7.1 |

[a]GAE, gallic acid equivalents
[b]CAE, caffeic acid equivalents
[c]Values reported as mean ± SE values (n = 3)

levels of total phenolic and hydroxycinnamic acid contents, we also tested these plant extracts for their total phenolic and hydroxycinnamic acid contents (Table-2). The highest levels of total phenolics were detected in both *Solanum* species, followed by *G. atroviridis* > *A. jiringa* > *C. sativa*. In addition, the highest levels of hydroxycinnamic acids were detected in both *Solanum* species, while the lowest level of hydrocycinnamic acids was detected in *C. sativa*.

Overall, all plant extracts tested exhibit good radical scavenging activities. *S. torvum*, with the highest total phenolic and hydroxycinnamic acid contents, was also found to exhibit the most powerful radical scavenging capability. In general, due to the additional protections provided by outer membranes[15] and efflux pumps[16], it is more difficult to inhibit Gram-negative bacteria, compared to their Gram-positive counterparts. Interestingly, *G. atroviridis* exhibited moderate (> 10 mm) to strong (> 20 mm) inhibition activities against all Gram-negative and Gram-negative bacterial strains tested. Notable, its inhibition against Gram-negative *P. aerugionosa* is comparable to that observed with commercial tetracycline. *C. sativa* exhibit no inhibition activity against all bacterial strains tested in this study, consistent with its low phytochemical contents. The low phytochemical contents observed with *C. sativa* could potentially due to the choice of extraction solvent and the use of other extraction solvents with different polarities may help to increase the phytochemical yields from *C. sativa* in future study.

## Conclusion

In conclusion, our study have determined and compared the bioactivities and corresponding phytochemcal coentents of five dietary plants. Based on our study, *S. torvum* was found to be a good source of antioxidants, while *G. atroviridis* could potential yield promising therapeutic agents with broad ranges of antibacterial activities.

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