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Full Length Research Paper

Phytochemicals, antioxidant and antimicrobial activity of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii* leaves

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Many exotic vegetables remain uninvestigated as potential sources of bioactive nutrients. The objective was to determine and compare nutrients, selected phytochemicals and antioxidant and antimicrobial activities in three types of leafy vegetables and a spice, including *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera,* and *Murraya koenigii*. Proximate analysis, vitamin C, beta-carotene, phenolics and flavanoids contents including antioxidant capacity and antimicrobial activity were determined. Soluble dietary fiber, vitamin C and lutein contents were highest in the leaf of *H. sabdariffa* including high contents of flavanoids and phenolic acids. *M. oleifera* leaf had the highest contents of carotenoids, (-) epicatechin and o-coumaric acid while *M. koenigii's* leaf had the highest content of benzoic acid. The anti-radical power values were 1.03, 0.63, 0.41, and 1.19 mg DPPH/mg extract for the ethanolic extracts from *C. asiatica, M. koenigii, H. sabdariffa, and M. oleifera* leaves, respectively. Total phenolic contents of the ethanolic extracts significantly affected their anti-radical power with a strong positive correlation between the total phenolic contents and their anti-radical power values. Antimicrobial activity of the three leaf extracts against Listeria *monocytogenes*, *Salmonella typhimurium*, and *E. coli* O157:H7 resulted in *H. sabdariffa* L. to exhibit the most antibacterial activity among all extracts evaluated in this study showing pathogen reduction to non-detectable limits. These leaves can be used as potential sources of vitamins, minerals, phenolics with antioxidant activity and as antimicrobial agents with potential medicinal value.

Key words: Phytochemicals, *Moringa oleifera, Hibiscus sabdariffa, Murraya koenigii,* antioxidant, antimicrobial.

INTRODUCTION

Vegetables are important sources of dietary fiber, carotenoids, vitamins, minerals, and phenolics for both infants and adults. Increased intake of vegetables is generally associated with a reduced risk of cancer and cardiovascular diseases (Azevedo-Meleiro, and Rodriguez-Amaya, 2005; Kris-Etherton et al., 2002). This association is based on the presence of different phytochemicals like carotenoids, flavonoids and phenolic acids in vegetables, with either potential or proven beneficial effects on human health (Mattila and Hellstrom, 2007).

Leaves of *C. asiatica* L. (Pegaga.), *H. sabdariffa* L. (gongura), and *M. oleifera* L. (Drumstick) are used as vegetables comprehensively in Asia. *M. koenigii* L. (Curry leaf) is an important spice in Asia. *C. asiatica* L. belongs to the Umbelliferae family. The crude extracts of *C. asiatica* have been shown to have antifertility, antioxidant,

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anti-inflammatory, immunomodulating, antitumor, antiproliferative, radioprotective and antigenotoxic properties (Siddique et al., 2008; Wang et al., 2005). Curry leaf, native to the south Asian countries has been shown to contain essential oils, coumarins, terpenoids, carbazole alkaloids, vitamins, α-tocopherol, β-carotene, and lutein (Choudhury and Garg, 2007), with documented antioxidant (Ningappa et al., 2008) hypoglycemic (Math and Balasubramaniam, 2005), anticarcinogenic (Dasgupta et al., 2003), antimicrobial, and mosquitocidal activities (Ramsewak et al., 1999). *H. sabdariffa* (gongura), of the family Malvaceae, an annual dicotyledonous herbaceous shrub plant popularly known as 'Gongura' in Telugu, 'Pitwa' in Hindi or 'Pulicha keerai' in Tamil is native to Asia and Africa and is commonly used to make jellies, jams, and beverages. Different parts of this plant have been recommended as a remedy for various ailments such as hypertension, pyrexia and liver disorders (Essa et al., 2006). In traditional medicine, this plant has features useful in several applications, including an antidote for poisonous chemicals (acids, alkali, pesticides) and mushrooms (Essa et al., 2006). *M. oleifera* belonging to the single genus family Moringaceae is a small, fast-growing ornamental tree originally found in India. Root, bark, pods, and leaves of this tree are used in traditional medicine for the treatment of human diseases, whereby the leaves are enriched in vitamin A and C. Pods and young leaves of this plant are primarily used as a vegetable (Shanker et al., 2007).

The novelty of our study lies in the comprehensive bio-analysis of exotic leaves in terms of their component nutrients so that they can be used as natural and inexpensive treatment alternatives to synthetic antioxidant drugs. In addition, the study contributes to new information about these leaves being good sources of nutrients and as antimicrobials. In this study, selected phytochemicals and nutrients of three exotic leafy vegetables (*C. asiatica* L., *H. sabdariffa* L., and *M. oleifera* L) and a single spice leaf (*M. koenigii*) were determined and the leaves evaluated for source quality (that is. poor or good source). Although *C. asiatica* and *M. koenigii* have been shown to have antioxidant activity, information on the antioxidant activity of *H. sabdariffa* and *M. oleifera* is limited; therefore the antioxidant activities of all the leaf extracts were determined and compared with other leafy vegetables.

In addition to the impact on the health of consumers, food borne infection also has a significant economic impact on the food industry in the form of recalls of products due to both actual and potential contamination. Approximately 17 million pounds of RTE (Ready To Eat) meat products were recalled due to Salmonella contaminations in 2007 and approximately 2.7 and 2.6 million pounds of beef products was recalled for *E. coli* O157:H7 during 2007 and 2008 (FSIS/USDA, 2009). To overcome recalls caused due to pathogen contamination there is a need to explore and apply alternative strategies involving natural antimicrobial agents such as leaf extracts. Hence, leaf extracts were evaluated for antimicrobial activity against Listeria *monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7.

MATERIALS AND METHODS

Sample preparation for analyses

H. sabdariffa, *C. asiatica*, (leaves were harvested from the vegetables grown in Arkansas) *M. oleifera,* and *M. koenigii* (leaves were harvested from the vegetables grown in Florida) leaves were washed in water and freeze-dried (Model 25LE, The Virtis Co., Inc., Gardiner, N. Y., U.S. A.). The dried leaves were ground using a coffee grinder model KSM 2B (Gillette Canada, Mississauga, Ontario, Canada), and passed through a 60 mesh sieve (w. s. Tyler Inc., Mentor, Ohio, U.S.A.). These fine-ground samples were stored at 4ºC for further analysis.

Nutrients determination

Dietary fiber was determined by the method of AACC (1991), moisture content was determined by air drying, fat by Soxhlet extraction. protein by Kjedahl method, ash by incineration and minerals using an atomic Absorption spectrophotometer (AAS) (Spectro, Flame Module E, ,Germany) after acid digestion of samples (AOAC, 1990).

Vitamin C determination

Vitamin C content was determined by the colorimetric method of Jagota and Dani, (1982), using 10.0 g of freshly cut leaf samples (5 x 5 mm) that were homogenized with de-ionized water, filtered, and centrifuged at 3000 × g for 15 min (centrifuge model J2-21, Beckman, Fullerton, Calif., U.S.A.). The supernatant was used for analysis.

Carotenoid determination

For carotenoid determination, the method of Radi et al. (1997) with the following modification was used. Sample preparation involved homogenization with acetone, centrifugation, hexane fractionation of the supernatants and filtration before HPLC analysis. For HPLC, a TSK-GEL Super-ODS column (Supelco, Bellefonte, Pa., U.S.A.) column was used. The separation of carotenoids was based on the procedure described previously by Pupin et al. (1999), with a slight modification as follows: Isocratic elution at a flow rate 0.5 mL/min with acetonitrile: methanol: 1, 2-dichloroethane in the ratio (60:35:5, v/v/v) which contained BHT (0.1%) and ammonium acetate (1%, w/v) was used.

Total phenolics and flavonoids determination

Total phenolics content was determined by Folin-Ciocalteau method (Singleton and Rossi, 1965). The total phenolics content on dry weight basis (mg/g) was calculated as follows: (A×91.994-0.6132)/1000 (R^2=0.9950), where A was absorbance at 760 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram dry material.

For total flavonoids determination, the method of Jia et al. (1999) was used. To 2 mL leaf extract (300 gm leaf sample refluxed in 80 mL methanol for 12 h at 85ºC). 1 mL of 5% $NaNO_2$ was added to 2 mL extract. To this mixture, 1 mL of 10% $Al(NO_3)_3$ was added,

incubated for 6 min, and followed by addition of 10 mL of 4% NaOH. After incubating for 15 min at room temperature, absorbance was measured at 500 nm. Rutin (0.1 to 0.7 mg/mL) was used to plot the standard curve, and the following equation was used to calculate the total flavonoids content (mg g^{-1} dry basis): A×2.272-0.0228 (R^2=0.999), where A was absorbance at 500 nm. Total flavonoid content was expressed as rutin equivalents in milligram per gram dry material.

Individual phenolic acids and flavonoids determination

Twelve standard phenolic acids were profiled by HPLC (Horax et al., 2005). Refluxed solution of methanolic extract of ground leaves was filtered and subjected to HPLC analysis. Absorbance was monitored at 254 nm. The concentrations of phenolic acids in the sample were expressed as mg/g dry weight basis.

Water-ethanol extraction of total phenolics

Ethanol (80%) was used to extract phenolics from the dried leaf samples, based on our previous study. Ten grams of the samples in an Erlenmeyer flask connected to a condenser with water coolant were added with 200 mL of 80% ethanol. The dispersions were heated in a heating mantle (Combo Mantle, Glas-Col, LLC., Terre Haute, IN, U.S.A.) at 80ºC for 2 h. The dispersions were then vacuum-filtered to separate the extracted phenolics from the residue and the residues were re-extracted twice with 150 mL of 80% ethanol. The combined ethanolic extract was vacuum-distilled using rotary evaporator (Büchi Rotavapor model 011, Brinkman, Wesbury, NY, U.S.A.) to evaporate ethanol, followed by freeze-drying to remove the remaining water. The dried extracts were stored at 4ºC until further analysis.

Antioxidant activity by free radical scavenging assay

The antioxidant activities of the extracts were evaluated using a modified free radical scavenging assay (Brand-Williams et al., 1995) using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) as a free radical. The initial and remaining DPPH concentrations (C_{DPPH}) in the solution were calculated from a calibration curve as follows: C_{DPPH} = 7.99 x 10^{-2} x Abs_{515nm} + 0.00258. The anti-radical power values were also calculated as $1/EC_{50}$ (in mg DPPH/mg extract x 10^{-2}) to express the antioxidant activity of the extracts.

Antimicrobial activity

S. typhimurium, E. coli O157:H7 and L. monocytogenes broth culture preparation

Cultures of *E. coli* O157:H7, *L. monocytogenes,* and *S. typhimurium* were prepared from frozen stock cultures (-70ºC) by inoculating ten milliliters of BHI with a loop of the organism and incubated at 37ºC for 24 h with 200-rpm agitation using a New Brunswick scientific (Edison, N.J. USA) agitating incubator. After 24 h, 10 mL of fresh BHI was inoculated with 10 µL of this culture, vortexed, and incubated at 37ºC for 18 h, to ensure the bacterial culture was in the exponential growth phase, at 200-rpm agitation. For the broth-culture study, 10 mL of fresh BHI was inoculated with 20 µL of the 18 h-incubated cultures to obtain a concentration of approximately 2 x 10^6 CFU/mL. This concentration was chosen to give a final concentration of 10^6 CFU/mL which is a high enough concentration to show a noticeable decline in the case of inhibitory action, but low enough to increase noticeably in the absence of inhibitory activity.
C. asiatica L. (Pegaga.), *H. sabdariffa* L. (Gongura) and *M. oleifera* L. (Drumstick) dried ethanolic extracts were solubilized in BHI at 10, 20, 40, and 80 mg/mL. One half milliliter of these solutions was combined with one half milliliter of the bacterial culture solutions for final extract concentrations of 5, 10, 20, and 40 mg/mL. These solutions were incubated at 37ºC for 24 h with 200-rpm agitation using a New Brunswick scientific (Edison, N.J. USA) agitating incubator. After 24 h, the extract/bacterial cultures were plated onto nutrient agar and incubated at 37ºC for 48 h.

Broth culture

Dried leaves ethanolic extracts were solubilized in BHI at 10, 20, 40, and 80 mg/mL. One half milliliter of these solutions was combined with one half milliliter of the bacterial culture solutions for final extract concentrations of 5, 10, 20, and 40 mg/mL. These solutions were incubated at 37ºC for 24 h with 200-rpm agitation using a New Brunswick Scientific (Edison, N.J. USA) agitating incubator. After 24 h, the extract/bacterial cultures were plated onto nutrient agar and incubated at 37ºC for 48 h.

Disc-diffusion method

Cultures of *E. coli* O157:H7, *L. monocytogenes*, and *S.* typhimurium were grown in BHI for 24 h. The cultures were swabbed onto nutrient agar plates with sterile swabs. The swabs were dipped into the bacterial cultures and pressed to the edge of the tube to remove excess liquid. Nutrient agar plates were streaked, turned 90º, streaked again and finally streaked diagonally to the first two streaks. The outside diameter of the agar was the swabbed. Discs (6 mm) were placed in the center of the streaked plates and 10 µL of the gongura solutions was pipette onto them following the method of O'Bryan (O'Bryan et al., 2008). After adding the gongura solutions, the discs were allowed to diffuse and absorb for 30 min. The plates were then incubated for 24 h at 37ºC. The zones of inhibition were measured by measuring the diameter of the inhibition zone at right angles and averaging.

Chicken meat model preparation

Raw and cooked boneless/skinless chicken breast meat was trimmed of excess fat and cut into approximately 1-cm^3 pieces (1 to 2 g). The most effective leaf extracts having antimicrobial activities (based on the broth culture study) was solubilized in PBS at 10, 20, and 40 mg/mL. The antibacterial efficacy of the leaf extract(s) on chicken breast meat was assessed. Since both *E. coli* O157:H7 and *S. typhimurium* are typically associated with raw products and *L. monocytogenes* with RTE products, raw chicken were challenged with *E. coli* O157:H7 and *S. Typhimurium* and cooked chicken was challenged with *Listeria monocytogenes.* For the raw chicken, the chicken pieces were immersed in the leaf extract solutions and allowed to drip-dry for 5 min followed by inoculation with bacterial suspensions by dipping the treated breast meat pieces and allowing them to drip-dry again. For cooked chicken, the chicken pieces were first inoculated with the bacterial suspensions followed by immersion in the leaf extract solutions. For both application methods the chicken was bagged into sterile sampling bags and stored at 4ºC for 12 days. The chicken was serial diluted in PBS and plated onto selective agar every 3 days.

Statistical analysis

Each analysis was performed in triplicate and the means and standard deviations determined. Data were subjected to ANOVA followed by Tukey's HSD test to determine differences between

Table 1. Moisture, crude protein, lipid, dietary fiber and ash contents (%) of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii*[*].

Leaf type	Moisture	Ash	Crude fat	Crude protein	Dietary fiber		Total % nutrients
					Soluble	Insoluble	
Hibiscus sabdariffa	7.8±13^{A}	6.5±3^{A}	3.1±1^{A}	17.3±2^{A}	8.5±7^{A}	34.7±19^{A}	77.9
Centella asiatica	8.0±4^{A}	12±1^{Ba}	2.9±3^{A}	19.4±5^{B}	0.8±2^{B}	26.3±3^{B}	69.4
Moringa oleifera	7.6±2^{B}	11.2±1^{C}	7.1±3^{B}	24.4±6^{C}	4.5±1^{C}	27.5±18^{B}	82.3
Murraya koenigii	6.8±4^{C}	12.5±3^{Bb}	5.1±3^{C}	11.8±3^{D}	2.5±6^{D}	38.8±7^{C}	77.5

*Values were means ± SD of triplicate samples; a, b: p<0.05. A, B, C, D: p<0.01.Comparisons with different letters (corresponding uppercase and lowercase) were done among *H. sabdariffa*, *C. asiatica*, *M. oleifera* and *M. koenigii*.

Table 2. Mineral contents (mg/g dry weight basis) of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii.*

Leaves	*Hibiscus sabdariffa*	*Centella asiatica*	*Moringa oleifera*	*Murraya koenigii*
P	2.16^{a}	2.90^{ab}	3.54^{b}	3.64^{b}
K	12.27^{a}	31.82^{b}	15.83^{c}	21.50^{d}
Ca	13.65^{a}	12.83^{a}	25.60^{b}	20.89
Mg	2.05^{a}	3.45^{b}	6.0^{c}	3.14^{b}
S	1.92^{a}	4.35^{b}	10.95^{c}	3.42^{b}
Na	0.07^{a}	1.76^{b}	0.36^{c}	0.052a
Fe	0.05^{a}	0.06^{ab}	0.073^{b}	0.05^{a}
Mn	0.44^{a}	0017^{b}	0.021^{c}	0.01c
Zn	0.03^{a}	0.07^{b}	0.031^{ac}	0.02^{c}
Cu	0.01^{a}	0.01^{b}	0.019^{C}	0.01^{b}
Al	0.047^{a}	0.055^{a}	0.094^{b}	0.07^{ab}

*Values were means ± SD of duplicate samples; a, b, c: P<0.05; Comparisons with different letters (corresponding uppercase and lowercase) were done among *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii*.

results. All analyses were conducted using JMP version 7.0.2, SAS Institute Inc.

RESULTS

Nutrients contents of *H. sabdariffa, C. asiatica, M. oleifera,* and *M. koenigii* leaves

Moisture, crude protein and fat, dietary fiber, and ash contents are shown in Table 1. *M. oleifera* leaf had the highest crude fat content (7.1%) among the four types of leaves and was much higher (3%) compared to that reported by Ruby et al. (2000), There were significant differences (p<0.01) among the crude protein contents of *H. sabdariffa* (17.3%), *C. asiatica* (19.4%), *M. oleifera* (24.4%), and *M. koenigii* (11.8%) leaves.

Mineral composition

The mineral composition of the leaves is shown in Table 2. Of the four types of leaves analyzed, *M. oleifera* had the highest amounts of Ca, Mg, S, Fe, Cu, and Al and *C. asiatica* had the highest concentrations of K, Na, Mn, and Zn. Though *M. koenigii* leaf's ash content was the highest among these four types of leaves, it's Mg, Fe, and Zn contents were not very high. The *H. sabdariffa* and *C. asiatica* leaves had higher Mn contents (0.44 and 0.17 mg/g) and relatively lower Al contents (0.047 and 0.055 mg/g) than *M. oleifera* and *M. koenigii* leaves.

Vitamin C, β-carotene and lutein contents

The contents of vitamin C, β-Carotene and lutein are shown in Table 3. There were significant (p<0.01) differences in the carotenoid and vitamin C levels among leaves of *H. sabdariffa*, *M. oleifera*, *C. asiatica*, and *M. koenigii* (Table 3). *H. sabdariffa* leaf had the highest vitamin C and lutein contents, which were 1.53 and 1.65 mg/g dry weight basis, respectively. *M. oleifera* leaf had the highest content of β-Carotene (6.65 mg/g dry weight basis) and *M. koenigii* leaf had the lowest contents of vitamin C (1.15 mg/g dry weight basis), β-Carotene (0.11 mg/g dry weight basis), and lutein (0.33 mg/g dry weight basis).

The vitamin C content of *C. asiatica* on dry weight

Table 3. Vitamin C, β-carotene and lutein contents (mg /g dry weight basis) of *Hibiscus sabdariffa, Centella asiatica, Moringa oleifera* and *Murraya koenigii*[*].

Leaf type	Vitamin C	β-Carotene	Lutein
Hibiscus sabdariffa	1.53.0±0.14[a]	0.55±0.03[ab]	1.65±0.10[a]
Centella asiatica	5.80±0.19[b]	0.32±0.01[b]	0.79±0.06[b]
Moringa oleifera	2.71±0.29[c]	0.66±0.05a	1.02±0.08[c]
Murraya koenigii	1.15±0.05[ab]	0.11±0.02[c]	0.33±0.07[d]

*Values were means ± SD of triplicate samples; a, b, c: P<0.05; Comparisons with different letters (corresponding uppercase and lowercase) were done among *H. sabdariffa*, *C. asiatica*, *M. oleifera* and *M.koenigii.*

Table 4. Total flavonoids, and phenolic contents (mg/g dry weight basis) of *Hibiscus sabdariffa, Centella asiatica, Moringa oleifera* and *Murraya koenigii*[*].

Leaves	Total flavonoids	Total phenolic
Hibiscus sabdariffa	77.802±2.717[a]	9.083±0.510[a]
Centella asiatica	79.312±2.787[a]	17.887±2.894[b]
Moringa oleifera	61.618±3.527[b]	15.356±3.960[ab]
Murraya koenigii	79.00±0.893[a]	14.547±1.214[ab]

*Values were means ± SD of triplicate samples; a, b: P<0.05; Comparisons were among *H. sabdariffa*, *C. asiatica*, *M. oleifera* and *M. koenigii.*

basis (5.80 mg/g) is higher than other vegetables including okra (0.26 mg per g), potato (0.22 mg/g), green bean (0.11 mg per g), broccoli (0.68 mg/g), spinach (1.10 mg/g) and pea (0.28 mg/g) (Pupin et al., 1999).

The β-carotene content of *M. oleifera* on fresh weight basis (0.158 mg/ g) was higher than that of endive (0.014 to 0.045 mg/g), spinach (0.039 to 0.05.5 mg/g) and kale (0.061 to 0.116 mg/g) (AACC, 1991; Radi et al., 1997).

Total flavonoids and phenolics contents

Total flavonoids and phenolics contents are included in Table 4. The total flavonoids content of *M. oleifera* leaf (61.61 mg/g dry weight basis) was significantly lower than the flavanoid contents of other leaves (p<0.01). However, no significant differences were observed among the total flavonoids contents of *H. sabdariffa*, *C. asiatica,* and *M. koenigii* leaves.

The total concentration of phenolic compounds in *C. asiatica* reported in his study was higher than in 28 kinds of fruits, grains, and vegetables (2.13 to 106 mg/g). In this study, *C. asiatica* leaf had the highest total phenolic content (17.88 mg/g dried basis), suggesting it was a good source of total phenolics.

Individual flavonoids and phenolic acids contents

Individual flavonoids and phenolic acid contents are presented in Table 5. With respect to the flavonoid and phenolic acid constituents, *H. sabdariffa* leaf was rich in naringin, rutin, syringic acid, and caffeic acid, which, on dry weight basis, were 15.79, 12.76, 5.21, and 3.44 mg/g, respectively. *C. asiatica* leaf was rich in naringin and caffeic acid, which, on dry weight basis, were 9.38 and 2.33 mg/g, respectively. *M. oleifera* leaf had the highest contents of (-) epicatechin (5.68 mg/g) and o-coumaric acid (6.45 mg/g) on dry weight basis, while *M. koenigii* leaf had the highest content of benzoic acid (11.37 mg/g dry weight basis).

Antioxidant activity

The total phenolics content, EC_{50}, and anti-radical power values of the ethanolic leaf extracts are shown in Table 6. Among the four leaves, ethanolic extracts of *M. oleifera* leaves had the highest phenolic content (122.40 mg/g). There were significant differences in anti-radical power among the ethanolic extracts from different leaves (p-value < 0.0001). The extract from *M. oleifera* had statistically the highest anti-radical power in comparison to other extracts and slightly similar to a known antioxidant, BHT. The anti-radical power values were 1.03, 0.63, 0.41, and 1.19 mg DPPH/mg extract for the ethanolic extracts from *C. asiatica, M. koenigii, H. sabdariffa, and M. oleifera* leaves, respectively.

Antimicrobial activity

The results of the effects of extracts of *H. sabdariffa*, *C. asiatica*, *M. oleifera,* and *M. koenigii,* on the inhibition of

Table 5. Individual flavonoids, and phenolic acid contents (mg/g dried basis) of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii*[*].

Leaves	*Hibiscus sabdariffa*	*Centella asiatica*	*Moringa oleifera*	*Murraya koenigii*
Rutin	12.76 ±0.356[a]	1.922 ±0.062[cba]	1.674 ±0.077[b]	2.357 ±0.034[cb]
Naringin	15.79 ±0.487[a]	9.382 ±1.443[b]	0.553 ±0.059[c]	0.326 ±0.027[c]
Caffeic acid	3.44 ±0.220[a]	2.333 ±0.151[b]	0.536 ±0.043[c]	Tr
Gentistic acid	0.93 ±0.066	ND	ND	ND
Syringic acid	5.21 ±0.260a	1.538 ±0.028[b]	ND	ND
(-)epicatechin	ND	ND	5.680 ±0.557	ND
ρ-coumaric acid	0.280 ±0.021[a]	0.458 ±0.025[b]	ND	ND
Sinapic acid	0.208 ±0.013[ab]	0.404 ±0.027[ab]	ND	0.791 ±0.177[bc]
Benzoic acid	2.044 ±0.077[a]	ND	1.219 ±0.214[ab]	11.378 ±1.448[c]
o-coumaric acid	1.345 ±0.029[a]	0.897 ±0.111[b]	6.457 ±0.854[c]	ND
t-ferulic	ND	ND	ND	0.067 ±0.002

[*]Values were means ± SD of triplicate samples; a, b, c: P<0.05; Comparisons with different letters (corresponding lowercase) were done among *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii*. Tr: trace (less than 1 mg per 100 g dried basis). ND: not-detectable.

Table 6. Total phenolic contents (in mg per g extract), EC_{50} (efficient concentration in mg extract/mg DPPH), and anti-radical power (mg DPPH/mg extract) of ethanolic leaf extracts *.

Leaf extract	Total phenolic content		EC50		Anti-radical power	
Hibiscus sabdariffa	57.8±16.9	b	2.47±0.19	A	0.41±0.03	d
Centella asiatica	86.4±4.7	b	0.97±0.06	C	1.03±0.06	b
Moringa oleifera	122.4±18.6	a	0.84±0.03	C	1.19±0.04	a
Murraya koenigii	79.9±3.6	b	1.60±0.03	B	0.63±0.01	c
BHT			0.62±0.01		2.32±0.001	a
P-value	0.0019		<0.0001		<0.0001	

*Values are means ± SD of three separate determinations; Mean values in the same column with different letter are significantly different. BHT: Known antioxidant.

L. monocytogenes, *S. typhimurium* and *E. coli* O157:H7 are summarized in Table 7. *M. koenigii* extracts at 20 and 40 mg/mL, when challenged with *E. coli* O157:H7 resulted in statistically lower numbers of bacteria when compared with the control of no extract, although the bacterial levels after 24 h were approximately 3 log CFU/mL higher than the initial inoculums indicating no practical inhibition. *M. koenigii* extract at 10, 20 and 40 mg/mL resulted in statistically lower levels of *L. monocytogenes* compared to the control of no extract. At 10, 20, and 40 mg/mL *M. koenigii* extract, the number of *L. monocytogenes* was 2, 4, and 5.5 log CFU/mL, respectively, lower than the control of no extract. *M. oleifera* L extracts at 40 mg/mL resulted in statistically lower levels of *L. monocytogenes.* At 40 mg/mL, *M. oleifera* L extract the number of *L. monocytogenes* was 3.16 log CFU/mL lower than the control of no extract. *H. sabdariffa* L. exhibited the most antibacterial activity of all extracts evaluated in this study. *H. sabdariffa* L. at 40 mg/mL reduced all 3 pathogens to non-detectable limits (LOD = 10) after 24 h. At 20 mg/mL, *H. sabdariffa* L. reduced *L. monocytogenes* to non-detectable limits after 24 h. At 10 and 20 mg/mL, *H. sabdariffa* L. significantly reduced *E. coli* O157:H7 and *S. typhimurium* when compared to the control of no extract (p<0.05). Based on the effectiveness of *H. sabdariffa* L. in causing microbial inhibition, this leaf extract was selected for further evaluating its antimicrobial efficacy in chicken meat system.

The results of dipping chicken breast meat inoculated with *E. coli* O157:H7, *L.m*., and *S*.T. into *H. sabdariffa* L (gongura) solutions are summarized in Table 8. Although treatments of 2 and 4% gongura extract differed from the control by approximately 1 log CFU/g on days 6 and 9 against *S. typhimurium* (figure 1 a), no significant difference was seen between the control and any treatment on day 12.

DISCUSSION AND CONCLUSION

Nutrients contents of *H. sabdariffa, C. asiatica, M. oleifera,* and *M. koenigii* leaves

Our results showed that, *H. sabdariffa*, *C. asiatica,* and *M. oleifera* leaves are all good sources of protein and ash (minerals). The proximate analyses do not account for

Table 7. Antibacterial effect of leaf extracts at various concentrations after 24 h in broth cultures.

Leaves	mg/mL	Bacteria		
		E. *coli O157:H7*	***S. typhimurium***	**L. *monocytogenes***
Control		9.33±0.07 [A12]	9.83±0.26 [AB]	9.66±0.30 [AB]
Centella asiatica	5	9.21±0.10 [ABC]	9.88±0.34 [A]	9.29±0.24 [ABC]
	10	9.08±0.07 [ABCD]	9.34±0.23 [ABC]	9.74±0.19 [A]
	20	9.12±0.05 [ABCD]	9.37±0.11 [ABC]	9.65±0.12 [AB]
	40	9.02±0.01 [ABCD]	8.92±0.72 [BC]	9.04±0.04 [BCD]
Murraya koenigii L.	5	9.15±0.09 [ABCD]	9.61±0.40 [ABC]	9.46±0.19 [ABC]
	10	9.09±0.04 [ABCD]	9.17±0.03 [ABC]	7.58±0.17 [E]
	20	9.01±0.02 [BCD]	9.29±0.22 [ABC]	5.61±0.27 [G]
	40	8.96±0.12 [CD]	9.02±0.11 [ABC]	4.15±0.50 [H]
Hibiscus sabdariffa L.	5	9.10±0.09 [ABCD]	9.46±0.43 [ABC]	9.29±0.23 [ABC]
	10	8.83±0.19 [D]	8.67±0.19 [C]	8.60±0.16 [D]
	20	4.00±0.27 [E]	1.23±0.48 [D]	0.95±0.00 [I]
	40	0.95±0.00 [F]	0.95±0.00 [D]	0.95±0.00 [I]
Moringa oleifera L	5	9.33±0.02 [AB]	9.41±0.35 [ABC]	9.31±0.20 [ABC]
	10	9.22±0.14 [ABC]	9.12±0.08 [ABC]	9.00±0.12 [CD]
	20	9.08±0.03 [ABCD]	9.09±0.05 [ABC]	9.05±0.01 [BCD]
	40	9.07±0.04 [ABCD]	9.08±0.26 [ABC]	6.50±0.02 [F]

[1]Values were means ± SD of triplicate samples. [2]Values within columns that share the same letters were not found to be significantly different using Tukey's HSD comparison ($p<0.05$).

Table 8. Antibacterial effect of gongura extract at various concentrations after 24 h incubation in broth culture.

Leaves	mg/mL	Bacteria		
		E. coli O157:H7	***S. Typhimurium***	***L. monocytogenes***
Control		9.33±0.07 [12B]	9.83±0.26 [A]	9.66±0.30 [A]
Hibiscus sabdariffa L.	5	9.10±0.09 [AB]	9.46±0.43 [AB]	9.29±0.23 [A]
	10	8.83±0.19 [B]	8.67±0.19 [B]	8.60±0.16 [B]
	20	4.00±0.27 [C]	1.23±0.48 [C]	0.95±0.00 [C]
	40	0.95±0.00 [D]	0.95±0.00 [C]	0.95±0.00 [C]

[2]Values within columns that share the same letter are not significantly different at the $\alpha=0.05$ level.

100% contents and so we might expect to see some carbohydrates or some extraneous plant material in the leaves. Existing literature lacks such a comprehensive analysis done with such a combination of exotic leaves. The information obtained from these data can aid in selecting these leaf extracts as good sources of nutrients.

In this study, the protein contents in *H. sabdariffa* and *M. oleifera* leaves were higher than that reported in the literature (9.0% in *H. sabdariffa* leaf and 14.4% in *M. oleifera* leaf, respectively), (Ruby et al., 2000; Nnam and Onyeke, 2003). No literature on protein content of *C. asiatica* was available. The data differences between our results and literature results of crude fat and protein contents might be due to differences in location, environmental conditions and maturity stages. Among the four leaves (*C. asiatica, H. sabdariffa, M. koenigii and M. oleifera*) analyzed, the soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) contents were lowest in *C. asiatica* leaves (26.3 and 0.8%), and highest in *H. sabdariffa* leaves (8.5% for SDF) and *M. koenigii* leaves (38.8% for IDF). Significant ($p<0.01$) variation was observed in SDF contents among the four leaf samples. In the present investigation, the SDF values of *H. sabdariffa* were more than that previously reported (1.2%), while the SDF values of *M. koenigii* and *M. oleifera* was less than that reported in literature (2.9%) (Punna and Paruchuri, 2004). Total dietary fiber in *M. oleifera* in this study was more than that (19.5%) reported

by Oduro et al. (2008). The reasons for higher/lower SDF values of vegetable leaves may due to varietal and/or location and/or agroclimatic conditions of the soil where the plant were grown or possibly the method used for fiber determination.

Mineral composition

From the present study, the concentrations of Zn, Cu, and Fe in the leaves of *M. koenigii* were higher than that reported by Narendhirakannan et al. (2005), (3, 5 and 57 mg per g, respectively). The concentrations of Ca, P, and K in *M. oleifera* leaves of this study were higher than those determined by Ruby et al. (2000), (18.4, 1.4, and 19.5 mg per g, respectively) and Nnam et al. (2003) including concentrations of Fe and Na. The difference between our study and other literature might be due to the different varietals, location, and agroclimatic conditions of the soil.

The dry basis concentrations of Ca in *M. oleifera* and K in *C. asiatica* were higher than those of *S. africana* (0.63 and 0.89 mg per g), *A. hybridus* (0.69 and 0.68 mg per g), *C. occidentalis* (0.52 and 0.83 mg per g) and *C. amygdalina* (0.52 and 0.80 mg per g) reported by Aletor et al. (2002), but lower than those of kale reported by Lefsruda et al. (2006). The concentrations of other elements including P, Mg, Na, Fe, Zn, Mn and Cu in the four types of leaves determined in this paper are all lower than those of *S. Africana*, *A. hybridus*, *C. occidentalis* and *C. amygdalina* (Punna and Paruchuri, 2004). Those results showed that *H. sabdariffa*, *C. asiatica*, *M. oleifera* and *M. koenigii* are not good source of minerals.

Vitamin C, β-carotene and lutein contents

According to the results of this study, *C. asiatica* leaf is a good source of vitamin C and *M. oleifera* is a good source of β-carotene. Carotenoids are beneficial in preventing major health problems such as cancer, cardiovascular/coronary heart diseases, and other diseases due to their antioxidant activity (Yeum and Russell, 2002). It has been reported that vitamin C consumption could be able to prevent cancer by inhibiting the formation of N-nitroso compounds in the stomach and by stimulation of the immune system (Byers and Perry, 1992).

Total flavonoids and phenolics contents

The total flavonoids and phenolic acids determined by HPLC in *H. sabdariffa*, *C. asiatica, M. oleifera* and *M. koenigii* leaves were 42.03, 16.93, 16.11, and 14.91 mg g on dry weight basis, respectively. *H. sabdariffa* had the highest total flavonoids and phenolic acids contents among the four kinds of leaves. We do not observe similar results when using a colorimetric method (Table 4) to determine these constituents probably because of the different types of extracts, these constituents were extracted in for the different methods. When the phenolics were extracted from the leaves using 80% ethanol, the total phenolic contents in the phenolic extracts differed in comparison to the total phenolics of leaves extracted using methanol. The total phenolic contents in the ethanolic extracts were 86.40, 79.90, 57.80, and 122.40 mg/g extract for the ethanolic extracts from *C. asiatica, M. koenigii, H. sabdariffa and M. oleifera* leaves, respectively (Table 6). These differences could be caused by the difference in polarity between methanol and ethanol used for extracting phenolics from the leaves. Zainol et al. (2003) reported that C. asiatica leaf extract, on dry weight basis, contained the highest amount of phenolic compounds (81.3 to 117 mg per g), followed by its root (64.6 to 105 mg per g) while the lowest concentration was present in the petiole (32.3 to 49.1 mg per g).

Total phenolic contents of the ethanolic extracts significantly affected their anti-radical power (p-value < 0.0001). A strong positive correlation between the total phenolic contents of the ethanolic extracts and their anti-radical power values was observed (R2 = 0.832). A similar correlation between the total phenolic contents and antioxidant activities from various plant extracts has also been shown by Silva et al. (2006).

The antimicrobial activity of *H. sabdariffa* leaf extract has been shown to be significantly better compared to other leaves both in broth and chicken meat systems. The leaf extract can possibly used in place of synthetic chemicals currently being used to disinfect/preserve meat types without resulting in side effects. The antimicrobial nature of this leaf extract can also find applications in several food systems as an antimicrobial preservative which can also prolong shelf life stability.

In conclusion, three types of leafy vegetables including *H. sabdariffa*, *C. asiatica*, *M. oleifera,* and a spice leaf, *M. koenigii,* were analyzed for nutrients and selected phytochemicals. According to the results, *C. asiatica* is a very good source of vitamin C, flavonoids, potassium, and total phenolics. *M. oleifera* is a good source of minerals, and β-carotene. *H. sabdariffa* is a good source dietary fiber, β-carotene, and lutein in addition to contributing to significant antimicrobial effect. Among the leaf extracts, *M. oleifera* had the highest total phenolic content and anti-radical power. These plant extracts can help human nutrition through their bioactive constituents that can offer protection against cardiovascular disease, pathogenic microbes, osteoporosis and other age-related conditions.

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