IN VITRO BIOACTIVITIES AND PHYTOCHEMICALS CONTENT OF VEGETABLES FROM SABAH, MALAYSIA

Jualang A. G.*, Adznila E., How S. E.

Faculty of Science and Natural Resources, Universiti Malaysia Sabah,
88400 Kota Kinabalu, Sabah, Malaysia.

**Corresponding e-mail: azlanajg@ums.edu.my*

ABSTRACT. *This study aims to investigate potential of vegetables from Sabah with value-added benefits in nutraceuticals. Fifty-five samples of vegetables were collected from local market and tested for antioxidant activity using DPPH• assay. Four species with high DPPH• scavenging activity (>80%) which are Cosmos caudatus, Eryngium foetidum, Ipomoea batatas and Manihot esculenta Crantz were selected and subjected to different solvents extraction and tested to different scavenging assays (DPPH•, O_2• and NO•), protein kinase-phosphatase assay (GSK-3β, MKK1, and MSG5) and antibacterial tests. Ethanol extract of I. batatas (90.56%), boiled water extract of M. esculenta Crantz (62.77%) and extractable polyphenol extract of E. foetidum (50.93%) exhibits comparable scavenging activities to catechin for DPPH•, O_2• and NO•, respectively. Polyphenols, phenolic acids, flavonoids and proanthocynidins are detected in all extracts at concentration between 0.001 mg/g to 0.52 mg/g. The highest total polyphenols content (0.40±0.01 mg GAE/g), total phenolics content (0.52±0.01 mg GAE/g), total flavonoids content (0.13±0.01 mg CE/g) and total proanthocyanidins content (0.12±0 mg CE/g) were obtained in extractable polyphenols of Cosmos caudatus. No extracts were observed as inhibitor for GSK-3β, MKK1 and MSG5. Inhibition of Pseudomonas aeruginosa (8.0 mm to 12.3 mm) was only obtained in extractable polyphenols and ethanol extracts. Extractable polyphenols of E. foetidum exhibit the largest inhibition of Pseudomonas aeruginosa (12.3 mm).*

KEYWORDS. Antibacterial, Antioxidant, Antikinases, Antiphosphatases, *Cosmos caudatus*, *Eryngium foetidum*, *Ipomoea batatas*, *Manihot esculenta*

INTRODUCTION

Antioxidants are capable of scavenging free radicals that were shown to be linked to age related illnesses and a large number of other illnesses (Amol *et al*., 2013). Restriction of synthetic antioxidants usage which been suspected to promote carcinogenesis has lead research on effective antioxidants originated from natural resources especially that were used as folk medicine or food (Amol *et al*., 2013).

Vegetables are essential to human in providing nutrients and various phytochemicals (Odufuwa *et al*., 2013). Consumption of vegetables could aid the prevention of aging related diseases and cancer (Genkinger *et al*., 2004). Cancer is the leading cause of death in

worldwide and it happen due to misregulation of signal transductions (Lobbezoo *et al*., 2003; Ougolkov and Billadeau, 2006). Protein phosphorylation is a major event in the signal transduction pathway which involves alteration of the downstream proteins conformation namely as protein kinases and phosphatases. Protein kinases serve central regulators of growth, embryogenesis, cell death, differentiation, proliferation, stress responses and apoptosis (Nakagami *et al*., 2005). Protein phosphatases are important regulators in glycogen metabolism, cell signaling, learning and memory, act as positive regulators of many hormonal responses, protein synthesis, muscle contraction, carbohydrate metabolism, transcription and neuronal signaling (Watanabe *et al*., 2001; Bennett *et al*., 2006). Abnormalities in the control of kinases and phosphatases had been detected in various types of cancers (Weinberg, 2007). Antioxidant compounds isolated from plants such as polyphenols and flavonoids are commonly associated as preventive agents against cancer at early stage (Thomas, 2008; El-Sayed *et al*., 2013).

Vegetables also have been long used in folk medicine to treat infectious disease caused by microbial and these have been supported by the discovery of antimicrobial phytochemicals from vegetables (Thomas, 2008). Flavonoid is one of the antioxidant compounds that was suggested to posses antibacterial activities (Thomas, 2008; Ahmad *et al*., 2012). Vegetables especially green leafy vegetables are among the top four common daily diets by Malaysian (Karim *et al*., 2008). To date, even though vegetables offer various health benefits to humans, there is little information on the biological properties of vegetables originating from Sabah. Therefore, the objectives of this study is to determine the *in vitro* bioactivities (antioxidant, antikinases, antiphosphatases and antibacterial) and phytochemicals content of different solvents extraction of 55 vegetable samples from Sabah, Malaysia.

MATERIALS AND METHODS

Plant Materials

A total of 55 samples derived from 33 genera and 42 species were bought from the local market in Sabah and deposited in BORNEENSIS, Institute of Tropical Biology and Conservation (ITBC), Universiti Malaysia Sabah. Plant parts were washed thoroughly with tap water, air dried, powdered, weighed and stored in air-tight containers at room temperature for extraction purposes.

Sample Extraction

Extractable polyphenols extract. Five grams of sample were extracted with acidic methanol:water (50:50, v/v; pH2) as described by Saura-Calixto *et al*. (2007) in the tube and shaken thoroughly for 1 hour at room temperature. The tube was centrifuged at 2500*g* for 10 min and the supernatant was recovered. Twenty-mL of acetone:water (70:30, v/v) then was added to the residues, shacked and centrifuged to recover the supernatant. The combined

methanolic and acetonic extract (extractable polyphenols) was evaporated to remove solvents and then freeze dried. The dried extract was dissolved in methanol at 10 mg/mL and subjected to DPPH (1,1-diphenyl- 2-picryl-hydrazyl) assay. Selected vegetable species with high scavenging activity was subjected to different types of solvent extraction and then tested for their phytochemicals and, antioxidants, antikinase or antiphosphatase and antimicrobial activities at concentration 0.5 mg/mL.

Ethanol extract. Five grams of sample were extracted three times with 95% (v/v) ethanol as described by Jimoh *et al*. (2010). The extract was filtered using Whatman paper No.1 and further evaporated to dryness with a rotary evaporator at 40°C under reduced pressure.

Boiling water extract. Extraction was done as described by Gülçin *et al*. (2004) with some modifications. Hundred milliliters of distilled water were added to 5 g of samples before boiled at 100˚C and stirred (1000 rpm) for 15 minutes. Extract was filtered using Whatman paper No.1 and freeze dried.

Antioxidant Assays

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging activity. Three-hundred µM of DPPH stock solutions was prepared in methanol. Then, 760 µL of the stock solution was added to the 40 µL of extract/catechin. Final concentration of the samples and the catechin (positive control) in the mixture was 0.5 mg/mL. The mixtures were incubated at 37 ˚C for 30 minutes (Jeong *et al*., 2004) before absorbance was measured at 517 nm (Multiskan^{TM} Go, USA). The scavenging activity was calculated following the equation described by Kumar *et al*. (2008):

Superoxide radical (O_2•) scavenging activity. Superoxide radical scavenging activity was determined as described by Liu *et al*. (1997). About 750 µL of 300 µM NBT solutions and 750 µL of 936 µM NADH solutions were added to 20 µL of 10 mg/mL extracts. Then, the mixture was topped up with 0.1 M Tris-HCl buffer (pH 7.4) to a total volume of 3 mL. 750 µL of 120 µM PMS was added to start the reaction. The mixture was incubated at room temperature (25 ± 2°C) for 5 minutes and the absorbance was measured at 560 nm (Multiskan^{TM} Go, USA). Final concentration of the samples and the positive control (catechin) was 0.053 mg/mL.

Nitric oxide radical (NO•) scavenging activity. Nitric oxide radical scavenging activity was determined as described by Kumar *et al*. (2008). About 500 µL sodium nitroprusside (5 mM in phosphate buffer saline pH 7.4) was mixed with 500 µL of 0.1 mg/mL extracts. The mixtures were incubated at room temperature (25 ± 2°C) for 30 min. Then, Griess reagent (1% (w/v) sulphanilamide, 2% (v/v) phosphoric acid and 0.1% (w/v) *N*- 1-naphthylenediamine dihydrochloride) was added at equal volume to the extract mixtures for colour development before the absorbance measured at 546 nm (Multiskan^{TM} Go, USA). Final concentration of the samples and the positive control (catechin) was 0.025 mg/mL.

Determination of the Phytochemicals Content

Total polyphenols content. Total polyphenol assay was conducted as described by Hakiman and Maziah (2009). 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times) was added to 100 µL of 1 mg/mL extract and was left for 5 minutes. Then, 2.5 mL of 7 % (w/v) of sodium carbonate (Na_2CO_3) was added. The mixture was incubated in room temperature (25 ± 2°C) for one hour before the absorbance was measured at 725 nm (Multiskan^{TM} Go, USA). The total polyphenols content of the extract was expressed as mg gallic acid equivalent per gram of sample.

Total phenolic acids content. Total phenolic acids assay was determined as described by Hakiman and Maziah (2009) with minor modification. About 900 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added to 100 µL of 1 mg/mL extract. The mixture was mixed thoroughly. After 5 minutes, 1 mL of 7 % (w/v) Na_2CO_3 was added. Then, the mixture was diluted to 2.5 mL by adding 400 µL distilled water. The mixture was incubated at room temperature (25 ± 2°C) for 90 minutes and the absorbance was measured at 750 nm (Multiskan^{TM} Go, USA). The total phenolic acids content was expressed as mg gallic acid equivalent per gram of sample.

Total flavonoids content. Total flavonoids assay was determined as described by Hakiman and Maziah (2009) with minor modification. About 400 µL of distilled water was added to 100 µL of 1 mg/mL extract. After that, 30 µL of 5 % (w/v) sodium nitrite was added. After 5 minutes, 30 µL of 10% (w/v) aluminum chloride was added. After 6 minutes, the mixture was then added with 200 µL of 1 M sodium hydroxide and subsequently added with distilled water to a final volume of 1 mL. The absorbance was measured at 510 nm (Multiskan^{TM} Go, USA). The total flavonoids content was expressed as mg catechin equivalent per gram sample.

Total proanthocyanidins content. Total proanthocyanidins assay was conducted as described by Porter (1989) with minor modification. 1 mL of a freshly prepared vanillin solution (1 g/100 mL of 70 % (v/v) sulfuric acid) was added to 500 µL of 1 mg/mL extract and further incubated at room temperature (25 ± 2°C) for 15 minutes. The absorbance was measured at 500 nm (Multiskan^{TM} Go, USA) and the total proanthocyanidins content was expressed as mg catechin equivalent per gram sample.

Antikinase and Antiphosphatase Assay

Both MAPK Kinase (MKK1) and MAP Kinase Phosphatase (MSG5) yeast strains (Table 1) were incubated in broth culture at 28˚C with 220 rpm for 2 days for fermentation purposes and later were incubated at 28˚C for 5 days for screening assay (Watanabe *et al.,* 1995). The glycogen synthase kinase – 3 beta (GSK-3β) screening test employed a transformant of gsk-3 null mutant (Table 1) and conducted according to method described by Cheenpracha *et al*. (2009). The strain was incubated at both 37°C and 25°C for 5 days. All tested extracts were dissolved in respective solvents to a stock concentration of 100 mg/ml and 20 µl of sample aliquots were impregnated on sterile paper dish for the yeast screening assay. Zones of inhibition are measured for data analysis.

Table 1: Genotype of yeast strains used in various type of screening assay.

Screening assay	Strains	Genotype	References
MAP kinase (MKK1)	$MKK1^{P386}$	Transformant from wild type 1788 with mutant type nNV7- $MKK1^{P386}$(*GAL1p- MKK1^{P386}*). *MATa/MATa ura3/ura3 leu2/leu2 trp1/trp1 his4/his4 can1/can1* [pNV7-$MKK1^{P386}$].	Watanabe (1995)
MAPK phosphatase (MSG5)	$MKK1^{P386}$_MSG5	*Mat**a** GAL1p-MKK1P386::TRP1 ura3 leu2 trp1 his4 can1*[Pspg14-*MSG5*]	Watanabe (1995); Ho (2001)
GSK-3β	pKT10-GSK-3β	*MATα his3 leu2 ura3 trp1 ade2 mck1::TRP1 mds1::HIS3 mrk1yol128C::LEU2* [pKT10-GSK3b]	Andoh *et al*., (2000); Cheenpracha *et al*. (2009)

Antimicrobial Assay

Microorganisms such as fungi (*Candida albicans* and *Candida krusei*), gram negative bacteria (*Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pneumonia*) were tested in this study. Bacterial cultures were suspended in nutrient broth and incubated for 24 hours at 37°C, while for fungal were in potato dextrose broth and incubated for 24 hours at 28°C (Barbour *et al*., 2004). Inoculum was prepared by diluting the microbial suspension with the broth to 0.5 McFarland standards. Then, 200 µL of inoculum was added into 20 mL of media agar. The agar was poured gently into petri dishes and left to solidify at room temperature. All tested extracts were dissolved in respective solvents to a stock concentration of 100 mg/ml and 20 µl of sample aliquots were impregnated on sterile paper dish for the antimicrobial screening assay. Zones of inhibition are measured for data analysis.

Statistical Analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) using SPSS 14.0 version. The significant differences between the mean values were compared using Duncan Multiple Range Test (DMRT) at $p<0.05$.

RESULTS AND DISCUSSION

Previous studies have recognized that antioxidant compounds may play an important role in explaining the benefit of vegetable consumption and thus some of vegetables in Sabah were used in this study to assess their antioxidant activity. This will allow identification of locally available vegetables that has antioxidant capability and developed into value added food. DPPH• scavenging assay is widely used in measuring antioxidant capacity due to its simplicity, rapid and sensitivity (Prior *et al*., 2005; Rafat *et al*., 2010). The DDPH assay shows that although the some vegetable samples shared the same genus but the performance in DPPH• scavenging is different (Table 1). Given an example where only two out of five

species of *Ipomoea* (*Ipomoea batatas* and *Ipomoea batatas* var. star shaped leaf) have high DPPH• scavenging activity. This finding is in line with report by Rasineni *et al*. (2008) on *C. forskhoii*. This may due to the variation of phytochemicals content in response to differences in genetics and growing conditions (Kalt, 2005). Different part of the plant also exhibit different performance on DPPH• scavenging activity. Leaf extract has the highest DPPH• scavenging activity compared to the other parts of vegetable. The biosynthesis of polyphenols which play an important role as antioxidant is a water soluble glycosylated flavonoids and mainly stored in epidermal cell vacuoles whereas the hydroxycinnamic acids are bound to cell walls in leaves (Meyer *et al*., 2006). Of all tested vegetable samples, *Cosmos caudatus* (83.09 ± 3.22), *Eryngium foetidum* (82.63 ± 3.18), *Ipomoea batatas* (89.43 ± 0.37) and *Manihot esculenta* Crantz (84.50 ± 2.75) having a high scavenging performance and comparable to catechin (90.96 ± 1.09%). This finding is in line with reports by Andarwulan *et al*. (2010) and Nur Faezah *et al*. (2013). Interestingly, those species promote better performance as compared to *Centella asiatica* (66.07 ± 4.66) that reported containing asiaticoside and poses high antioxidant activity (Tatmiya *et al*., 2014), and therefore were subjected to different solvents extraction for further analysis.

Scavenging Activities of Selected Vegetables Extracted with Different Solvents

Solvents extraction such as acetone, methanol, ethanol and their combination with water are frequently used for extracting antioxidant compounds from plants (Sultana *et al*., 2009; Dai and Mumper, 2010). In general, methanol extract lower molecular weight (such as polyphenols) while acetone is efficient for higher molecular weight (such as flavanols) (Dai and Mumper, 2010). Meanwhile, ethanol is able to extract polyphenols and tannins, whereas waters extract polypeptides, anthocyanins and tannins (Cowan, 1999). Hence in this study, the air-dried samples were extracted with different solvents to yield extractable polyphenols (combine methanol and acetone), ethanol and boiling water extracts. These extracts were further tested for DPPH•, O_2• and NO• scavenging assays at concentration of 0.5 mg/mL, 0.053 mg/mL and 0.025 mg/mL, respectively. The result revealed that different extracting solvents have different antioxidants performance.

The DPPH• scavenging activitiy of selected vegetables species were ranging from 20.53% to 90.56% (Table 3). Ethanol extract of *I. batatas* exhibit the highest DPPH• scavenging activity (90.56 ± 0.24%) followed by extractable polyphenols of *I. batatas* (89.43 ± 0.37 %), ethanol extracts of *C. caudatus* (84.68 ± 0.29 %) and *E. foetidum* (75.87 ± 8.84 %). Water extract of *Ipomoea batatas* exhibit the lowest DPPH• scavenging activity (6.59 ± 0.66 %). This observation was similar to Moure *et al*. (2000) and Kaur and Kapoor (2002). Ethanol is an organic solvent that effective to extract bioactive compounds from plants and did not toxic to human (Prior *et al*., 2005; Sultana *et al*., 2009; Sulaiman *et al*., 2011). However, the present of total polyphenols, phenolic acids and flavonoids content in this extract are relatively lower (0.002-0.07 mg/g) as compared to extractable polyphenols (0.004-0.52 mg/g) that detailed in Table 4. This suggest that those compounds might not be the main contributor for DPPH• scavenging activity in *C. caudatus* and *I. batatas* in ethanol

extract or *C. caudatus* and *M. esculenta* Crantz in boiled water extract. Previous study has suggested that non-phenolic compounds such as vitamins and carotenoids are capable to act as radical scavenger and thus might be attribute to this variation (Rafat *et al*., 2010; Kaneria and Chanda, 2012).

Extractable polyphenols of *E. foetidum* exhibits the highest NO• scavenging activity (50.93 ± 4.48%) followed by extractable polyphenols of *I. batatas* (46.17 ± 4.75 %), ethanol extract of *I. batatas* (45.97 ± 3.89 %), extractable polyphenols of *M. esculenta* Crantz (45.27 ± 5.30 %) and extractable polyphenols of *C. caudatus* (44.31 ± 5.59 %). Water extract of *M. esculenta* Crantz exhibit the lowest NO• scavenging activity (9.45 ± 6.99 %). Dai and Mumper (2010) also reported that extraction using high polarity organic solvents resulting in an extract with considerably more effective radical scavengers than those with less polarity organic solvents. However, extractable polyphenols in *E. foetidum* relatively compose lower total polyphenols content (0.35 ± 0.01 %), total phenolic acids content (0.39 ± 0.02 %), total flavanoids content (0.08 ± 0.02 %) and total proanthocyanidins content (0.02 ± 0 %) as compared to extracteble polyphenols of *C. caudatus*, respectively. This suggesting that those compounds may not significantly responsible for NO• scavenging activity.

Generally, organic solvents exhibit high antioxidant activity but unlike DPPH• and NO• assays, boiled water extract of *M. esculenta* Crantz exhibits the highest O_2• scavenging activity (62.77 ± 1.56 %) followed by boiled water extracts of *E. foetidum* (48.94 ± 7.19 %), *C. caudatus* (42.62 ± 5.51 %), and *I. batatas* (38.55 ± 10.80 %). Ethanol extract of *M. esculenta* Crantz exhibit the lowest NO• scavenging activity (11.20 ± 4.45 %). Taubert *et al*. (2003) reported that pyrogallol group including proanthocyanidins exhibits highest superoxide scavenging activity. However, in this study the content of total proanthocyanidins content in boiled water extract is relatively lower (0.001-0.12 mg/g) suggesting that other compounds might be responsible for the scavenging activity (Santiago *et al*., 2014). Heat treatment applied on the boiled water extract is expected to degrade cell wall (Miglio *et al*., 2008) and to promote higher solubility of the active compounds by increasing the solubility and mass transfer rate. Heat reduced viscosity and surface tension of the solvents to reach sample matrices and improve the extraction rate (Sultana *et al*., 2009; Dai and Mumper, 2010).

It is commonly known that extract composed high antioxidant compounds will also has high antioxidant activity (Wong *et al*., 2006). However, in this finding although *C. caudatus* has the highest content of antioxidant compounds (total polyphenols, total phenolics, total flavanoids and total proanthocyanidins) but it does not exhibit high antioxidant activity for DPPH•, O_2• and NO• scavenging assays, respectively. This observation was in agreement report by Rafat *et al*. (2010). This variation might be due to the degree of polymerization and the interaction between the diverse chemical structures to the colorimetric assay which affecting the antioxidant capacity (Ismail *et al*., 2004; Sulaiman *et al*., 2011).

Table 2: DPPH• scavenging activities of extractable polyphenol extract of vegetables from Sabah

No.	Vegetable species (local name)	Part tested	DPPH• scavenging activity (%)
1	*Amaranthus tricolor* (Bayam merah hijau), *Lactuca sativa* (Sayur minyak), *Brassica rapa* subspecies *chinensis* var. wavy leaf (Sawi putih keriting), *Sechium edule* (Sayur janggut), *Lactuca sativa* L. var. longifolia (Bola-bola), *Amaranthus gangeticus* var. round leaf (Bayam merah), *Amaranthus oleraceus* (Bayam kampung), *Amaranthus tricolor* var. wavy leaf (Bayam keriting), *Petroselinum crispum (*Daun pasri)	Leaf/stem	0 – 10
2	*Talinum triangulare* (Sam choi), *Spinacia oleracea* (Bayam papai), *Amaranthus paniculatus* (Bayam putih), *Brassica chinensis* var. *parhinensis commanis* (Sawi manis), *Momordica charantia* (Pucuk peria), *Solanum nigrum* (Tutan), *Limocharis flava* (Tambung ambung), *Raphanus sativus* (Batang lobak putih), *Cucurbita pepo* L. (Pucuk labu merah), *Basella alba* L. (Gandula), *Monochoria vaginalis* (Tayaan), *Psophocarpus tetragonolobus* (Kacang belimbing), *Brassica chinesis* (Bunga jipun), *Sauropus androgynus* (Sayur manis), *Brassica juncea* (Sawi pahit), *Allium tuberosum* (Bunga kucai), *Brassica alboglabra* (Kailan), *Vigna unguiculata* (Daun kacang), *Brassica rapa* subspecies *chinensis* Tokyo bekana (Sawi keriting)	Leaf/stem/fruit	11 – 20
3	*Manihot esculenta* var. curly (Pucuk ubi keriting), *Crassocephalum crepidioides* (Rumpai merah), *Allium cepa* cv. Aggregatum (Daun bawang), *Carica papaya* L. (Betik), *Ipomoea aquatic* (Kangkung), *Sesbania grandiflora* (L.) Poir. (Kembang turi), *Brassica rapa* subspecies *chinensis* (Sawi putih), *Athyrium esculentum* (Pucuk paku), *Sesbania grandiflora* (L.) Poir. (Kembang turi), *Moringa oleifera* (Daun kilur), *Passiflora foetida* L. (Lapak-lapak), *Ipomoea batatas* (L.) Poir (Pucuk ubi manis)	Leaf/stem/ inflorescent	21 – 30
4	*Amaranthus gangeticus* var. oblong leaf (Bayam merah bukit), *Erechtites valerianifolia* (Sayur jipun), *Stenochlaena palustris* Bedd. (Lemiding), *Ipomoea batatas* 'Black heart' (Pucuk ubi manis merah bukit), *Carica papaya* L. (Betik)	Leaf/stem	31 – 40
5	*Oenanthe javanica* WC. (Daun selom), *Piper sarmentosum* Roxb. (Daun sirih), *Lycium chinensis* L. (Kiugi)	Leaf/stem	31 – 40
6	*Crassocephalum crepidioides* (Rumpai merah)	Root	51 – 60
7	*Centella asiatica* (Pegaga), *Ipomoea batatas* var. green star shaped leaf (Pucuk ubi manis)	Leaf/stem	61 – 70
8	*Cosmos caudatus* (Ulam raja), *Eryngium foetidum* (Bawing), *Ipomoea batatas* (Pucuk ubi rambat), *Manihot esculenta* Crantz (Pucuk ubi kayu)	Leaf/stem	81 – 90
9	Catechin (positive control)		90.96 ± 1.09

Table 3: Antioxidative activities of selected vegetables species extracted with different solvents.

Sample	DPPH• scavenging activity (%)			O_2• scavenging activity (%)			NO• scavenging activity (%)		
	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water
Cosmos caudatus	83.09 ± 3.22^{a}	84.68 ± 0.29^{c}	76.83 ± 2.15^{c}	22.98 ± 8.95^{a}	21.01 ± 7.28^{cd}	42.62 ± 5.51^{ab}	44.31 ± 5.59^{a}	44.29 ± 3.23^{b}	41.66 ± 5.08^{d}
Eryngium foetidum	82.63 ± 3.18^{a}	75.87 ± 8.84^{b}	20.53 ± 0.99^{b}	33.67 ± 5.67^{b}	17.34 ± 6.92^{bc}	48.94 ± 7.19^{b}	50.93 ± 4.48^{b}	38.71 ± 8.78^{ab}	26.14 ± 2.45^{c}
Ipomoea batatas	89.43 ± 0.37^{b}	90.56 ± 0.24^{d}	6.59 ± 0.66^{a}	18.44 ± 8.77^{a}	27.21 ± 7.50^{d}	38.55 ± 10.80^{a}	46.17 ± 4.75^{ab}	45.97 ± 3.89^{b}	17.19 ± 2.57^{b}
Manihot esculenta Crantz	84.50 ± 2.75^{a}	26.58 ± 4.07^{a}	75.54 ± 0.59^{c}	20.19 ± 4.28^{a}	11.20 ± 4.45^{ab}	62.77 ± 1.56^{c}	45.27 ± 5.30^{ab}	34.18 ± 13.64^{ab}	9.45 ± 6.99^{a}
Catechin (positive control)	90.96 ± 1.09^{b}	90.96 ± 1.09^{d}	90.96 ± 1.09^{d}	41.00 ± 9.69^{c}	41.00 ± 9.69^{e}	41.00 ± 9.69^{ab}	64.18 ± 1.43^{c}	64.18 ± 1.43^{c}	64.18 ± 1.43^{e}

Values are mean ± standard deviation of three replicates. ***DPPH• -****tested at the concentration of 0.5 mg/mL.* ***O_2• -*** *tested at the concentration of 0.053 mg/mL.* ***NO• -*** *tested at the concentration of 0.025 mg/mL. Mean values between plant samples were compared using Duncan's multiple range test at $p<0.05$.*

Table 4: Phytochemicals content of selected vegetables species extracted with different type of solvents

Sample	Total polyphenols content (mg GAE/g sample)			Total phenolics acid content (mg GAE/g sample)			Total flavonoids content (mg CE/g sample)			Total proanthocyanidins content (mg CE/g sample)		
	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water	Extractable polyphenols	Ethanol	Boiled water
Cosmos caudatus	0.40 ± 0.01^{c}	0.05 ± 0^{c}	0.06 ± 0^{c}	0.52 ± 0.01^{c}	0.05 ± 0^{c}	0.07 ± 0^{c}	0.13 ± 0.01^{c}	0.01 ± 0^{a}	0.014 ± 0^{b}	0.12 ± 0^{d}	0.01 ± 0^{c}	0.01 ± 0^{c}
Eryngium foetidum	0.35 ± 0.01^{bc}	0.03 ± 0^{b}	0.02 ± 0^{a}	0.39 ± 0.02^{b}	0.04 ± 0^{b}	0.02 ± 0^{a}	0.08 ± 0.02^{b}	0.02 ± 0^{b}	0.004 ± 0^{a}	0.02 ± 0^{b}	0.01 ± 0^{c}	0.002 ± 0^{b}
Ipomoea batatas	0.31 ± 0.02^{ab}	0.07 ± 0.01^{d}	0.02 ± 0^{a}	0.41 ± 0.01^{b}	0.06 ± 0^{d}	0.02 ± 0^{a}	0.12 ± 0.01^{c}	0.02 ± 0^{b}	0.004 ± 0^{a}	0.004 ± 0^{a}	0.002 ± 0^{a}	0.002 ± 0^{b}
Manihot esculenta Crantz	0.27 ± 0.02^{a}	0.02 ± 0^{a}	0.03 ± 0^{b}	0.29 ± 0.01^{a}	0.02 ± 0^{a}	0.03 ± 0^{b}	0.04 ± 0.01^{a}	0.01 ± 0^{a}	0.002 ± 0^{a}	0.04 ± 0^{c}	0.004 ± 0^{b}	0.001 ± 0^{a}

Values are mean ± standard deviation of three replicates. GAE: garlic acid equivalent. CE: catechin equivalent. Mean values between plant samples were compared using Duncan's multiple range test at $p<0.05$.

Antikinase and Antiphosphatase Activities of Selected Vegetables Species

Free radicals such as reactive oxygen species (ROS) have been associated with a wide array of human diseases including cancer (Waris and Ahsan, 2006). ROS caused an indirect oxidative damage on DNA through lipid peroxidation and affecting cytoplasmic and nuclear signal transduction pathways (Matés and Sánchez-Jiménez, 2000; Waris and Ahsan, 2006). Interference of ROS on signal cascade system such as mitogen activated protein kinases (MAPKs) has led to carcinogenesis (Matés and Sánchez-Jiménez, 2000).

MKK is a cytoplasmic protein kinase that binds specifically to MAPK as activator (Brunet *et al*., 1999). $MKK1^{P386}$ is a hyperactive mutation of MAPK Kinase (MKK1) that has proline instead of serine at the position 386. In galactose media, the presence of galactose will induce the GAL1 promoter which resulting in the overexpression of this mutant gene thus suppressing both the Pkc1 and Bck1 deletion and inhibit the growth of the yeast. As a result, in the present inhibitor will cause yeast growth on galactose media but not on glucose media (Watanabe *et al*., 1995; Pang *et al*., 2009). $MKK1^{P386}$ inhibitor can either target on MKK1 or MPK1 in the PKC1 pathway thus MAP Kinase Phosphatase (MSG5) screening test was carried in order to specify the inhibition (Watanabe *et al*., 1995; Pang *et al*., 2009). MSG5 is a protein tyrosine phosphatase that belongs to a novel subclass of protein phosphatases whose substrates is MAP kinase family members (Doi *et al*., 1994). In the MSG5 screening test, $MKK1^{P386}$-MSG yeast able to grow on both glucose and galactose media thus inhibitory activity for MKK1 is confirmed if no yeast growth on galactose media but growing on glucose media (Doi *et al.,* 1994; Watanabe *et al*., 1995). Meanwhile, glycogen synthase kinase-3 (GSK-3) is a highly conversed protein kinase that involved significantly with diverse physiological process such as cancer (Ougolkov and Billadeau, 2006), diabetes (Ross *et al*., 1999) and neurological disorders (Eldar-Finkelman, 2002). GSK-3 is an unusual protein kinase where it is normally active in cells and it is primarily regulated through the inhibition of its activity (Doble and Woodgett, 2003). In addition to that, compared with the other protein kinases, GSK-3 preference for primed substrates which are previously phosphorylated by another kinase (Doble and Woodgett, 2003). Potential inhibitor will caused the inhibition of the yeast incubated on 37°C due to the *gsk-3* null mutant yeast exhibits temperature sensitivity and no significant activity on 25°C (Andoh *et al*., 2000). However, in this study none of the extracts have inhibition activity towards kinase or phosphatase screening assays.

Antimicrobial Activities of Selected Vegetables Species

Nine microbial species tested in these screening assays which are *Candida albicans, Candida krusei*, *Staphylococcus aureus* and *Streptococcus pneumonia*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Those microbes have long history caused a wide variety of human diseases and some are capable of producing highly toxic compounds that adversely affect human health (You, 2006). The results from the tests (Table 5) showed that only *P. aeruginosa* was inhibited by the extracts. This finding is in contrast to Rasdi *et al*. (2010) where the ethanol extract of *C. caudatus* has the ability to inhibit *E. aerogenes* and *C. albican*; and Noumedem *et al*. (2013) on methanol extract of *M. esculenta* Crantz that inhibit growth of *E. aerogenes*. The variation in this result might be due to the differences in the solvent, extraction system, growth condition and plant maturity that might affect the results (Faiza *et al*., 2013).

Pseudomonas aeruginosa is a versatile gram-negative bacterium and one of the top three leading causes of human infection due to its intrinsic resistance to antibiotics and disinfectants (Stover *et al*., 2000). Antibacterial activity of the tested extracts ranging from 8.0 mm to 12.3 mm. Inhibition of bacteria exhibited by both extractable polyphenols and ethanol extracts but not in boiled water extract. Extractable polyphenols of *E. foetidum* exhibits the largest inhibition (12.33 ± 0.47 mm) followed by ethanol extract of *E. foetidum* (11.33 ± 0.47 mm), and extractable polyphenols and ethanol extract of *I. batatas* (9.00 ± 0 mm). The lowest inhibition of *P. aeruginosa* was observed on extractable polyphenols and ethanol extract of *C. caudatus* (8.00 ± 0 mm). This finding is in agreement with report by Ahmad *et al*. (2012) and Faiza *et al*. (2013). The antibacterial properties might be due to the availability of phenolics and flavonoids compounds that able to disrupt bacterial DNA replication (Jayalakshmi *et al*., 2013).

Table 5: Antibacterial activity of selected vegetables species extracted with different type of solvents

Sample	Type of solvents	*Pseudomonas aeruginosa*
Cosmos caudatus	Extractable polyphenols	8.00 ± 0^{b}
	Ethanol extract	8.00 ± 0^{b}
	Boiled water extract	0^{a}
Eryngium foetidum	Extractable polyphenols	12.33 ± 0.47^{f}
	Ethanol extract	11.33 ± 0.47^{e}
	Boiled water extract	0^{a}
Ipomoea batatas	Extractable polyphenols	9.00 ± 0^{d}
	Ethanol extract	9.00 ± 0^{d}
	Boiled water extract	0^{a}
Manihot esculenta Crantz	Extractable polyphenols	8.33 ± 0.47^{bc}
	Ethanol extract	8.67 ± 0.47^{cd}
	Boiled water extract	0^{a}
Penicillin	Positive control	14.67 ± 0.47^{g}

Values are mean ± standard deviation of triplicate analyses. All samples were tested at the concentration of 100 mg/mL. Positive control was tested at the concentration of 1% (w/v), respectively. Extractable polyphenol is the combination of methanol and acetone extracts. Means in each column with different alphabets are significantly different tested using Duncan's multiple range test at $p<0.05$.

CONCLUSION

A total of 55 vegetable samples were screened for their antioxidant activity using the DPPH• scavenging assay. Four selected species with high DPPH• scavenging activity that are *Cosmos caudatus, Eryngium foetidum, Ipomoea batatas* and *Manihot esculenta* Crantz were further extracted with different solvents system. Ethanol extract of *I. batatas*, extractable polyphenols of *E. foetidum* and boiled water extracts of *M. esculenta* Crantz exhibits higher scavenging activity towards DPPH•, O_2• and NO•, respectively. Polyphenols, phenolic acids, flavonoids and proanthocynidins are observed in all extracts, however, the highest total polyphenols content, total phenolics content, total flavonoids content and total proanthocyanidins content were obtained in extractable polyphenols of *C. caudatus*, indicating that these phytochemicals may not significantly contribute to the scavenging

activity in ethanol extract of *I. batatas*, boiled water extract of *M. esculenta* Crantz and extractable polyphenols of *E. foetidum*. No extracts observed as inhibitor to GSK-3β, MKK1 and MSG5, while inhibition of *Pseudomonas aeruginosa* was obtained only on extractable polyphenols and ethanol extracts. This finding has suggested that vegetables found in Sabah have value-added benefits for human health as an alternative for nutraceutical product development.

ACKNOWLEDGMENTS

Thanks to Mr. Julius Kulip, a botanist at Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, for species identification.

REFERENCES

Ahmad, F., Hasan, I., Chishti, D. K. and Ahmad, H. 2012. Antibacterial activity of *Raphanus sativus* Linn. seed extract. *Global Journal of Medical Research,* **12**(11): 25-34

Amol, R. K., Tarkasband, Y. S. and Nambiar, V. V. 2013. *In vitro* antioxidant activity of *Kirganelia reticulata* stem. *Advance Research in Pharmaceuticals and Biologicals,* **3**(II): 408-413.

Andarwulan, N., Batari, R., Sandrasari, D. A., Bolling, B. and Wijaya, H. 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry,* **121**: 1231-1235.

Andoh, T., Hirata, Y. and Kikuchi, A. 2000. Yeast glycogen synthase kinase 3 is involved in protein degradation in coorperation with *Bul1*, *Bul2* and *Rsp5*. *Molecular and Cellular Biology,* **20**(18): 6712-6720.

Barbour, E. K., Al Sharif, M., Sagherian, V. K., Habre, A. N., Talhouk, R. S. and Talhouk, S. N. 2004. Screening of selected indigenous plants of Lebanon for antimicrobial activity. *Journal of Ethnopharmacology,* **93**: 1-7.

Bennett, D., Lyulcheva, E. and Alphey, L. 2006. Towards a comprehensive analysis of the protein phosphatase 1 interactome in *Drosophilla*. *Journal of Molecular Biology*, **364**: 196-212.

Brunet , A., Roux. D., Lenormand, P., Dowd, S., Keyse, S. and Pouysségur, J. 1999. Nuclear translocation of p42/p44 Mitogen-Activated Protein Kinase is required for growth factor-induced gene expression and cell cycle entry. *The EMBO Journal,* **18**(3): 664-674.

Cheenpracha, S., Zhang, H., Mar, A.M., Foss, A.P., Foo, S.H., Lai, N.S., Jee, J.M., Seow, H.F., Ho, C.C. and Chang, L.C. 2009. Yeast glycogen synthase kinase-3beta pathway inhibitors from an organic extract of *Streptomyces* sp. *Journal of Natural Product,* **72**(8): 1520-1523.

Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Reviews,* **12**(4): 564-582.

Dai, J. and Mumper, R. J. 2010. Review plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules,* **15**: 7313-7352.

Doble, B. W. and Woodgett, J. R. 2003. GSK-3: Tricks of the trade for a multi-tasking kinase. *Journal of Cell Science,* **116**: 1175-1180.

Doi, K., Gartner, A., Ammerer, G., Errede, B., Shinkawa, H., Sugimoto, K. and Matsumoto, K. 1994. MSG5, a novel protein phosphatase promotes adaptation to pheromone response in *S. cerevisiae*. *The EMBO Journal,* **13**(1): 61-70.

Eldar-Finkelmen, H. 2002. Glycogen synthase kinase 3 : An emerging therapeutic target. *Trends in Molecular Medicine,* **8**(3): 126-132.

El-Sayed, M. M., Abdel-Aziz, M. M., Abdel-Gawad, M. M., Abdel-Hameed, E. S., Ahmed, W. S. and Abdel-Lateef, E. E. 2013. Chemical constituents and cytotoxic activity of *Cassia glauca* Lan. leaves. *Life Science Journal,* **10**(3): 1617-1625.

Faiza, R., Waqas, K. K., Adeel, M. and Muhammad, G. 2013. Detection of bioactive fractions of *Justicia adhatoda* L. leaves. *Canadian Journal of Applied Sciences,* **1**(3): 388-398.

Genkinger, J. M., Platz, E. A., Hoffman, S. C., Comstock, G. W. and Helzlsouer, K. J. 2004. Fruit, vegetable, and antioxidant intake and all-cause, cancer, and cardiovascular disease mortality in a community-dwelling population in Washington country, Maryland. *American Journal of Epidemiology,* **160**(12): 1223-1233.

Gülçin, İ., Sat, İ. G., Beydemir, Ş., Elmastaş, M. and Küfrevioğlu, Ö. I. 2004. Comparison of antioxidant activity of Clove (*Eugenia caryophylata* Thunb) buds and Lavender (*Lavandula stoechas* L.). *Food Chemistry,* **87**: 393-400.

Hakiman, M. and Maziah, M. 2009. Non enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea a*ccessions. *Journal of Medicinal Plants Research,* **3**(3): 120-131.

Ho, C. C. 2001. *Molecular Cell Biology, Biodiversity and Biotechnology.* Kota Kinabalu: University Malaysia Sabah.

Ismail, A., Marjan, Z. M. and Foong, C. W. 2004. Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry,* **87**: 581-586.

Jayalakshmi, B., Raveesha, K. A. and Amruthesh, K. N. 2013. Evaluation of antibacterial and antioxidant potential of *Euphorbia cotinifolia* Linn. leaf extracts. *Chemical Industry and Chemical Engineering Quarterly,* **1**: 99-99.

Jeong, S. M., Kim, S. Y., Kim, D. R., Jo, S. C., Nam, K. C. and Ahn, D. U. 2004. Effect of heat treatment on the antioxidant activity of extracts from citrus peels. *Journal of Agricultural and Food Chemistry,* **52**: 3389-3393.

Jimoh, F. O., Adedapo, A. A. and Afolayan, A. J. 2010. Comparison of the nutritional value and biological activities of the acetone, methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonorus*. *Food and Chemical Toxicology,* **48**: 964-971.

Kalt, W. 2005. Effects of prodcution and precessing factors on major fruits and vegetable antioxidants. *Journal of Food Science,* **70**(1): R11-R19.

Kaneria, M. and Chanda, S. 2012. Evaluation of antioxidant and antimicrobial properties of *Manilkara zapota* L. (Chiku) leaves by sequential soxhlet extraction method. *Asian Pacific Journal of Tropical Biomedicine,* S1526-S1533.

Karim, N. A., Safiah, M., Jamal, K., Siti Haslinda, Zuhaida, H., Rohida, S., Fatimah, S., Siti Norazlin, Poh, B. K., Kandiah, M., Zalilah, M. S., Wan Manan, W. M., Fatimah, S. and Azmi, M. Y. 2008. Food consumption patterns: Findings from the Malaysian Adult Nutrition Survey (MANS). *Malaysian Journal of Nutrition,* **14**(1): 25-39.

Kaur, C. and Kapoor, H. C. 2002. Antioxidant level and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology* **37**: 153-161.

Kumar, K. S., Ganesan, K. and Rao, P. V. S. 2008. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) – an edible seaweed. *Food Chemistry,* **107**: 289-295.

Liu, F., Ooi, V. E. and Chang, S. T. 1997. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences,* **60**(10): 763-771.

Lobbezoo, M., Giaccone, G. and Kalken, C. 2003. The oncologist meeting report: signal transduction modulators for cancer therapy: from promise to practice. *The Oncologist* **8**: 210-213.

Matés, J. M. and Sánchez-Jiménez, F. M. 2000. Review role of reactive oxygen species in apoptosis: implication for cancer therapy. *The International Journal of Biochemistry and Cell Biology,* **32**: 157-170.

Meyer, S., Cerovic, Z. G., Goulas, Y., Montpied, P., Demotes-Mainard, S., Bidel, L. P. R., Moya, I. and Dreyer, E. 2006. Relationships between optically assessed polyphenols and chlorophyll contents and leaf mass per area ratio in woody plants: a signature of the carbon-nitrogen balance within leaves? *Plant, Cell and Environment,* **29**: 1338-1348.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V. and Pellegrini, N. 2008. Effects of different cooking methods on nutritional and physiochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry* **56**: 139-147.

Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M. J. and Lema, J. M. 2000. Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *Journal of Agricultural and Food Chemistry,* **48(9)**: 3890-3897.

Nakagami, H., Pitzschke, A. and Hirt, H. 2005. Emerging MAP kinase pathways in plant stress signaling. *Trend in Plant Sciences*, **10**(7): 340-346.

Noumedem, J. A. K., Mihasan, M., Lacmata, S. T., Stefan, M., Kuiate, J. R. and Kuete, V. 2013. Antibacterial activities of the methanol extracts of ten Cameroonian vegetables against gram negative multidrug resistant bacteria. *BMC Complementary and Alternative Medicine,* 13-26.

Nur Faezah, O., Siti Aishah, H. and Umi Kalsom, Y. 2013. Comparative evaluation of organic and inorganic fertilizers on total phenolic, total flavonoid, antioxidant activity and cyanogenic glycosides in cassava (*Manihot esculenta*). *African Journal of Biotechnology,* **12**(18): 2414-2421.

Odufuwa, Temitope, K., Atunnise, Adeleke, Kinnah, Joseph, H., Adeniji, Salau, P. O. and Adewale, B. 2013. Changes in saponins content of some selected Nigerian vegetables during blanching and juicing. *Journal of Environmental Science, Toxicology and Food Technology,* **3**(3): 38-42.

Ougolkov, A. V. and Billadeau, D. D. 2006. Targeting GSK-3: A promising approach for cancer therapy?. *Future Oncology,* **2**(1): 91-100.

Pang, K. L., Thong, W. L. and How, S. E. 2009. *Cinnamomum iners* as Mitogen Activated Protein Kinase Kinase (MKK1) Inhibitor. *International Journal of Engineering and Technology,* **1**(4): 310-313.

Porter, L. J., Hrstich, L. N. and Chan, B. G. 1986. The conversion of proanthocyanidins and prodelphenidins to cyanidins and delphenidins. *Phytochemistry,* **25**: 223-230.

Prior, R. L., Wu, X. L. and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* **53**: 4290-4302.

Rafat, A., Philip, K. and Muniandy, S. 2010. Antioxidant potential and phenolic content of ethanolic extract of selected Malaysian plants. *Research Journal of Biotechnology* **5**(1): 16-19.

Rasdi, M. N. H. Othman, A. S., Sule, A. B. and Ahmed, Q. U. 2010. Antimicrobial studies of *Cosmos caudatus* Kunth. (Compositae). *Journal of Medicinal Plants Research,* **4**(8): 669-673.

Rasineni, Girish, K., Dayananda, S. and Attipalli, R. 2008. Free radical quenching activity and polyphenols in three species of *Colues*. *Journal of Medicinal Plants Research,* **2**(10): 285-291.

Ross, S.E., Erickson, R. L., Hemati, N. and MacDougald O. A. 1999. Glycogen Synthase Kinase-3 is an insulin-regulated C/EBPá kinase. *Molecular Cell Biology,* **19**(12): 8433–8441.

Santiago, L. A., Dayrit, K. C., Correa, P. C. B. and Mayor, A. B. R. 2014. Comparison of antioxidant and free radical scavenging activity of triterpenesα-amyrin, oleanolic acid and ursolic acid. *Journal of Natural Products,* **7**(2014): 29-36.

Saura-Calixto, F., Serrano, J. and Goñi, I. 2007. Intake and bioaccessibility of total polyphenols in a whole diet. *Food Chemistry,* **101**: 492-501.

Stover, C. K., Pham, X. Q., Erwin, A. L., Mizoguchi, S. D., Warrener, P., Hickey, M. J., Brinkman, F. S. L., Hufnagle, W. O., Kowalik, D. J., Lagrou, M., Garber, R. L., Goltry, L., Tolentino, E., Westbrock-Wadman, S., Yuan, Y., Brody, L. L., Coulter, S. N., Folger, K. R., Kas, A., Larbig, K., Lim, R., Smith, K., Spencer, D., Wong, G. K. S., Wu, Z., Paulsen, I. T., Reizer, J., Saler, M. H., Hancock, R. E. W., Lory, S. and Olsen, M. V. 2000. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. *Nature,* **406**: 959-964.

Sulaiman, S. F., Abu Bakar Sajak, A., Kheng, L. O., Supriatno, Eng, M. S. 2011. Effects of solvents in extracting polyphenols and antioxidants of selected raw vegetables. *Journal of Food Composition and Analysis,* **24**: 506-515.

Sultana, B., Anwar, F. and Ashraf, M. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules,* **14**: 2167-2180.

Tatmiya, R.N., Kiran , S.C., Vibhuti, M.J. and Vrinda, S.T. 2014. Screening of proper leaf size in *Centella asiatica* for antioxidant potential and separation of phenolics using RP-HPLC. *J. App. Pharm Sci.* **4**(02): 43-47.

Taubert, D., Breitenbach, T., Lazar, A., Censarek, P., Harlfinger, S., Berkels, R., Klaus, W. and Roesen, R. 2003. Reaction rate constrans of superoxide scavenging by plant antioxidants. *Free Radical Biology & Medicine,* **35**(12): 1599-1607.

Thomas, S. C. L. 2008. *Vegetables and Fruits Nutritional and Therapeutic Values*. United States of America: CRC Press.

Waris, G. and Ahsan, H. 2006. Review reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis,* **5**(14): 1-8.

Watanabe, T., Huang, H., Horiuchi, A., da Cruz Silva, E. F., Hsieh-Wilson, L., Allen, P. B., Shenolikar, S., Greengard, P. and Nairn, A.C. 2001. Protein phosphatase 1 regulation by inhibitors and targeting subunits. *PNAS*, **98**(6): 3080-3085.

Watanabe, Y., Irie, K. and Matsumoto, K. 1995. Yeast *RLM1* encodes a serum response factor like protein that may function downstream of the Mpk1 (Slt2) mitogen activated protein kinase pathway. *Molecular and Cellular Biology,* **15**(10): 5740-5749.

Weinberg, R. A. 2007. Cancer-principles and overview. *In:* Lewin, B., Cassimeris, L., Lingappa, V. R. and Plopper, G. (eds.). *Cells*. Jones and Bartlett Publishers, USA.

Wong, S. P., Leong, L. P. and Koh, J. H. W. 2006. Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* **99**(4): 775-783.

You, X. 2006. Food safety and food additive of antiseptic. *Food Science and Technology* **1**: 1-4.