Food Research 5 (3) : 262 - 272 (June 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Anti-*salmonellosis* agent for foodborne illness from *Mangifera odorata* (kuini) extracts

[1,*]Adnan, H., [1]Ismail, N., [1]Hassan, H. and [2]Mat-Ali, M.S.

[1]*Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor*
[2]*Governance and Legal Centre, Malaysian Agricultural Research and Development Institute (MARDI), MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor*

Article history:
Received: 8 October 2020
Received in revised form: 9 November 2020
Accepted: 21 January 2021
Available Online: 6 June 2021

Keywords:
Salmonella,
Pathogen,
Mangifera odorata,
Seed kernel,
Peel and flesh

DOI:
https://doi.org/10.26656/fr.2017.5(3).574

Abstract

Salmonellosis infection *caused by Salmonella* bacteria is a public endemic problem in Malaysia with long-term morbidity and mortality effects. Thus, this study aimed to explore the antipathogenic activity of natural extracts from *Mangifera odorata* against two *Salmonella* species causing *Salmonellosis.* The extracts were derived from peel, flesh, and kernel seed of *M. odorata*. The inhibition performance of the extracts against both *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Enteritidis bacteria were subsequently tested by using a bioassay-guided fractionation method. Results showed that the extracts derived from the kernel seed had the highest inhibition percentage of 83-90% against the *Salmonellosis* infection, followed by the peel with an inhibition of 61-67%, and lastly the flesh with an inhibition of 53-69%. The inhibition activities of hexane extracted flesh (FCH), methanol extracted peel (PCM), and methanol treated kernel seed (KTM) against *S. enterica* ser. Typhimurium bacteria were 59, 67 and 83%, respectively. Furthermore, the *S. enterica* ser. Enteritidis bacteria were found to be highly susceptible against the methanol extracted kernel seed (KCM), followed by the hexane extracted peel (PCH) and flesh (FTH) with the inhibition percentage of 90, 69 and 59%, respectively. The highly active anti-*Salmonellosis* performance of *M. odorata* extracts was attributed to its intrinsically high total phenolics content at 8-10 g GAE/g extract, high ferric reducing antioxidant power value (FRAP) at 18-22 g Fe^{2+}/g extract and excellent scavenging activity with the inhibition performance ranges between 86% and 90%. This study revealed the antipathogenic activity of methanol extracts of *M. odorata* kernel seed inhibited the growth of both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis *bacteria.* This study also discovered the prophylactic property of natural compounds in *M. odorata* kernel seed extracts and could be used as an anti-*Salmonellosis* agent. In the near future, *M. odorata* can be developed as an innovative functional food source for specific groups that are vulnerable to *Salmonellosis*.

1. Introduction

Foodborne illness is a major concern of many food safety issues in Malaysia. The children, pregnant women, elderly and immune-compromised individuals are among the vulnerable groups being affected by foodborne diseases. *Salmonella* is one of the most common bacterial pathogens that caused foodborne illnesses such as food poisoning with symptoms of diarrhoea, high fever and focal infection. *Salmonellae* infections are one of the many foodborne diseases, where severe infection could lead to gastroenteritis, enteric fever, bacteraemia, focal disease, and death (World Health Organization, 2018).

Salmonellae infection is one of the public illnesses in Malaysia (Mohan *et al*., 2019). The latter outbreak was reported due to contaminated bubble pudding (The Sun Daily, 2020) while the former incidence was the presence of *Salmonella enterica* serovar Enteritidis in a contaminated net pancake ('roti jala') (Packierisamy *et al*., 2018). At present, the food poisoning rate in Malaysia had increased significantly from 2005 to 2013 (A'aishah, 2014) and the cases continued to rise to 24% in 2018 (Ministry of Health, 2019). In the foodborne outbreaks, serovar *Enteritidis* was reported to be the

*Corresponding author.
Email: *hazniza@mardi.gov.my*

eISSN: 2550-2166 /

most frequently isolated bacteria followed by Typhimurium. The most common food vehicles associated with Enteritidis serovars are eggs, chicken, pork, beef, whereas Typhimurium serovars are mainly hosted in chicken, leafy greens and peanut butter (Andino and Hanning, 2015; Afshari *et al*., 2018). Treatment for *Salmonellosis* infection using drugs or steroids may weaken the immune system and ingestion of antibiotic could eliminate the good bacteria in the body and make the immune system unable to fight off infections.

This paper focused on the investigation of plant extracts from *Mangifera odorata* as an anti-salmonellosis agent against both *S. enterica* ser. Enteritidis and *S. enterica* ser. Typhimurium bacteria. The *M. odorata* or locally known as *kuini* is one of the mango family, which mango is a good source of natural products such as polyphenols and phenolic acids (Brandt *et al.,* 2004), terpenoids (Ediriweera *et al*., 2017) and carotenoids (Lasano *et al*., 2019). Since ages, mango has a long history in folk medicine. The *M. indica* fruit, leaves, root and bark have been used as ingredients in traditional medicine to treat diseases such as diarrhoea, ulcer, pneumonia and bowel disorder (Muthu *et al*., 2006; Khandare, 2016). However, the antipathogen activity such as anti-*Salmonellosis* of *M. odorata* has not yet been reported elsewhere. In the phytochemical analyses of wild *Mangifera* species, *M. odorata* contains applicable amounts of antioxidants and total phenolic content (Salahuddin *et al*., 2016). These findings indicated that the bioactivity of *M. odorata* may contain antibacterial property. Thus, the objective of this study was to identify active extracts of *M. odorata* against *S. enterica* ser. Enteritidis and *S. enterica* ser. Typhimurium.

In this study, the *M. odorata* fruit parts such as flesh, peel, and kernel seeds were prepared as dried sample before extracted into three different groups of extracts. The polarity-dependent extracts were tested against the pathogenic bacteria using a bioassay-guided fractionation method. The antipathogen activity was measured as the percentage of bacterial inhibition from a mean of triplicate readings. Next, extract with inhibition efficacy of more than 50% was reported as active whereas the lesser was reported as ineffective (Adnan *et al*., 2017).

2. Materials and methods

2.1 Preparation of Mangifera odorata samples

Matured fruits of *M. odorata* at stage 5 of maturity index were washed and air-dried. The fruits were separated into two groups. The first group samples were untreated and served as control. Another group of samples were treated with blanching. The fruit was peeled and sliced before blended into a puree. The seed was removed from the kernel before cut into small pieces. The flesh puree, peel and seed kernel were dried separately in an oven dryer at 40°C for 2 days. The final moisture content of all samples was approximately 10% (w/w). All flesh, peel and seed kernel samples including both control and treated samples were blended into fine powders before packed, sealed and labelled separately in an aluminium bag.

2.2 Preparation of extracts

All samples (flesh, peel and kernel seed) were extracted using a bioassay-guided fractionation method. Each sample was extracted separately by using different solvents in sequences such as *n*-hexane, ethyl acetate, and methanol to give non-polar, medium polar and polar extracts respectively. Each extraction was conducted twice by using a sonicator (DECON F5100b, UK) at 25°C for an hour. All elutes were filtered using a qualitative filter paper (125 mm; Fisher UK) and concentrated using a rotary evaporator (Buchi R-205, Switzerland) under reduced pressure before dried under nitrogen flow to remove excess solvent. All extracts were kept sealed in glass containers and stored at -20°C.

2.3 Inhibition of pathogenic bacteria

The antibacterial activity of each sample was evaluated using a disc diffusion method as reported by Adnan *et al*. (2017). The analysis was performed with two types of pathogenic bacteria namely *S. enterica* ser. Typhimurium (ATTC 53648) and *S. enterica* ser. Enteritidis (MDC15). Briefly, 200 µL of the fresh bacterial assay was pipetted and spread evenly onto a Mueller-Hinton (MH) plate agar to produce a uniform bacteria lawn. A diameter of 6 mm sterilised disc was embedded onto the bacteria lawn. Each disc was pipetted with 10 µL of extract (1 mg/mL). The plates were incubated overnight at 37°C. The inhibition activity of pathogenic bacteria was measured as the diameter (Ø, mm) of clear zone surrounding the disc. The antipathogenic activity was expressed in term of inhibition percentage, where extract with more than 50% of inhibition can be regarded as an active extract. The working concentration of each extract was prepared at 1 mg/mL using dimethyl sulfoxide (Sigma-Aldrich, US) before analysis. The antibiotic tetracycline (Chopra and Robert, 2001) at 1 mg/mL was used as a positive control, whereas dimethyl sulfoxide was used as a negative control.

2.4 Determination of total phenolic content (TPC)

The TPC of each sample was determined based on Folin–Ciocalteau assay method from Singleton and Rossi (1965) with gallic acid (Sigma, Germany) as a standard phenolic compound. Briefly, 50 µL of each extract solution and standard gallic acid solutions (12.5, 25.0, 50, 100, and 200 µg/mL) was mixed with 50 µL of distilled water in a 96-wells plate. Then, 100 µL of Folin -Ciocalteau reagent (Ajax Finechem, US) solution (1:10 dilution) was added into the mixture. Approximately after 6 mins, 100 µL of 7.5 % (w/v) sodium carbonate solution was added into the mixture before kept in dark for two hours. The absorbance of the mixture was measured at 765 nm using the 96-wells microplate reader (Biotek Gen5, Vermont, US) by using distilled water as a blank. The TPC was expressed in term of a gram of gallic acid equivalents (g GAE/g extract) based on the concentrations of standard gallic acid used. The data were reported as mean ± standard deviation of three replicates.

2.5 Determination of antioxidant activities

2.5.1 Determination of free radical scavenging activity

The free radical scavenging activity of each extract was measured following a marginally modified method of Blois (1958) using spectrophotometric assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a stable radical reagent. The hydrogen atom or electron-donating ability of each sample and pure compound was measured from the bleaching of a purple coloured methanol solution of (DPPH) reagent. Briefly, 100 µL of the extract was added into 200 µL of 0.007% methanol solution of DPPH. After 40 mins of dark incubation period under room temperature, the absorbance of each mixture was recorded against a blank at 517 nm using a 96-wells microplate reader (Biotek Gen5, Vermont, US). Trolox is a well-known standard with strong antioxidant activities was used as a positive control in this work. The inhibition percentage of free radical DPPH in the extract was calculated as follow:

$$\text{Inhibition (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

2.5.2 Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as described previously by Benzie and Strain (1996) that measured the reduction ability of ferric plasma under a low pH value. The ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) was reduced to ferrous form (Fe^{2+}-TPTZ) with intense blue colour. To prepare the FRAP reagent, 10 volumes of 300 mM acetate buffer (pH 3.6) was mixed with one volume of 10 mM TPTZ in 40 mM HCl and one volume of 20 mM $FeCl_3 \cdot 6H_2O$. Then, the mixtures were incubated at 37°C for about 10 mins. Following, 20 µL of extract solution and 80 µL of distilled water were added into 200 µL of freshly prepared FRAP reagent using a 96-wells plate (Biotek Gen5, Vermont, US). After four mins, the absorbance of each mixture was recorded using a 96-wells microplate reader at 593 nm against reagent blank. The reagent blank was prepared by using the same procedure as described above, except for the extract solution which was substituted with 20 µL of distilled water. A calibration curve was plotted by using ferrous sulphate as a standard with the concentration ranges from 12.5 to 200 µg/mL. Referring to the calibration curve, the FRAP value was calculated and expressed in term of a gram of Fe^{2+} equivalents per g of extracts (g Fe^{2+} /g extract).

2.6 Statistical analysis

The statistical data for antipathogenic activity were analysed using Minitab® 16.1.1 Statistical Analyses (U.K). All data values were expressed as mean ± standard deviation (SD) and analysed using one-way ANOVA with *P* values of < 0.05. Dunnett's method was used to compare the mean of the sample and control. Unless stated, Turkey Method was used to compare all of the possible pairwise difference of means. Other analyses as such total phenolic content and antioxidant activities were analysed using the Statistical Analysis Software (SAS) package (ver. 9.4 of SAS Institute, Inc. Cary, NC, 2008). Data were mean ± standard deviation (SD) and analysed using one-way ANOVA with P values of < 0.05. The Duncan Multiple Range Test (DMRT) was used to compare the significant differences between the means.

3. Results

3.1 Antipathogen activity of Mangifera odorata kernel seed extracts

Figure 1 (A) shows the antipathogen activity of *M. odorata* kernel seeds extracts. The methanol treated kernel seed (KTM) exhibited the highest (83%) antipathogenic activity against *S. enterica* ser. Typhimurium. On the contrary, both ethyl acetate extracts from control (KCE) and treated kernel seed (KTE) were active but showed a lower (53-57%) inhibition activity. The antipathogen activity of potent (KTM) extracts against *S. enterica* ser. Typhimurium was significantly different (P< 0.05), whereas both active extracts of (KCE) and (KTE) were not significantly different (P< 0.05). The methanol extracts from control kernel seed (KCM), *n*-hexane extracts from control (KCH), and treated kernel seed (KTH) were found as inactive inhibitor against *S. enterica* ser. Typhimurium.

Interestingly, almost all kernel seed extracts were active against *S. enterica* ser. Enteritidis. Results indicated that both methanol extracts of control (KCM) and treated kernel seed (KTM) were very potent (87-90%) in inhibiting the dispersion of *S. enterica* ser. Enteritidis. In contrast, all kernel seed extracts (KCH, KTH and KTE) were active and exhibited between 57-61% inhibition activity. The antipathogen activities between the potent (KCM) and (KTM) extracts against *S. enterica* ser. Enteritidis however were not significantly different (P<0.05). Similarly, all active extracts of (KCH), (KTH) and (KTE*)* were not significantly different (P<0.05). Only the ethyl acetate extract of the control kernel seed (KCE) was inactive against *S. enterica* ser. Enteritidis.

3.2 Antipathogen activity of Mangifera odorata peel extracts

Figure 1 (B) shows the extracts derived from the control peel were more active in inhibiting the *S. enterica* ser. Typhimurium than the treated peel. In the context of inhibiting *S. enterica* ser. Typhimurium, the methanol extract of control peel (PCM) had the highest antipathogenic activity of 67%, followed by the ethyl acetate extract of control peel (PCE) with an antipathogenic activity of 58%, and lastly the hexane extract of treated peel (PTH) with an antipathogenic activity of 53%. The antipathogen activities among the three (PCE, PCM and PTH) extracts against *S. enterica* ser. Typhimurium were significantly different (P< 0.05).

In the context of inhibiting *S. enterica* ser. Enteritidis, the hexane extract of control peel (PCH) had the highest antipathogenic performance of 69%, while the methanol extract of control peel (PCM) exhibited a moderate (64%) antipathogenic activity. Both hexane (PTH) and methanol (PTM) extracts of treated peel had a moderate antipathogenic activity of 53-57 %. In contrast, both ethyl acetate extracts of control (PCE) and treated peel (PTE) were found inactive to inhibit *S. enterica* ser. Enteritidis. The antipathogen activities between (PCH) and (*PCM*) extracts against *S. enterica* ser. Enteritidis were significantly different (P< 0.05), however activities between (PTH) and (PTM*)* extracts were not significantly different (P< 0.05).

3.3 Antipathogen activity of Mangifera odorata flesh extracts

Figure 1 (C) indicates that all hexane and ethyl acetate extracts (FCH, FCE, FTH and FTE) from control and treated flesh were active inhibitors against *S. enterica* ser. Typhimurium and showed inhibition between the percentage of 57-59%. The antipathogen activities among all those extracts against *S. enterica* ser. Typhimurium however were insignificantly different (P< 0.05). In contrast, both methanol extracts of control (FCM) and treated flesh (FTM) were found inactive to inhibit *S. enterica* ser. Typhimurium.

Despite all other extracts were found inactive against the *S. enterica* ser. Enteritidis*,* the hexane extracts of treated flesh (FTH) demonstrated an inhibition activity of 59%. Results indicated that most of the flesh extracts exhibited satisfactory inhibition performances against *S. enterica* ser. Typhimurium*,* but failed to demonstrate inhibition activity against *S. enterica* ser. Enteritidis. This finding suggested that the *S. enterica* ser. Typhimurium is more susceptible to the flesh extracts as

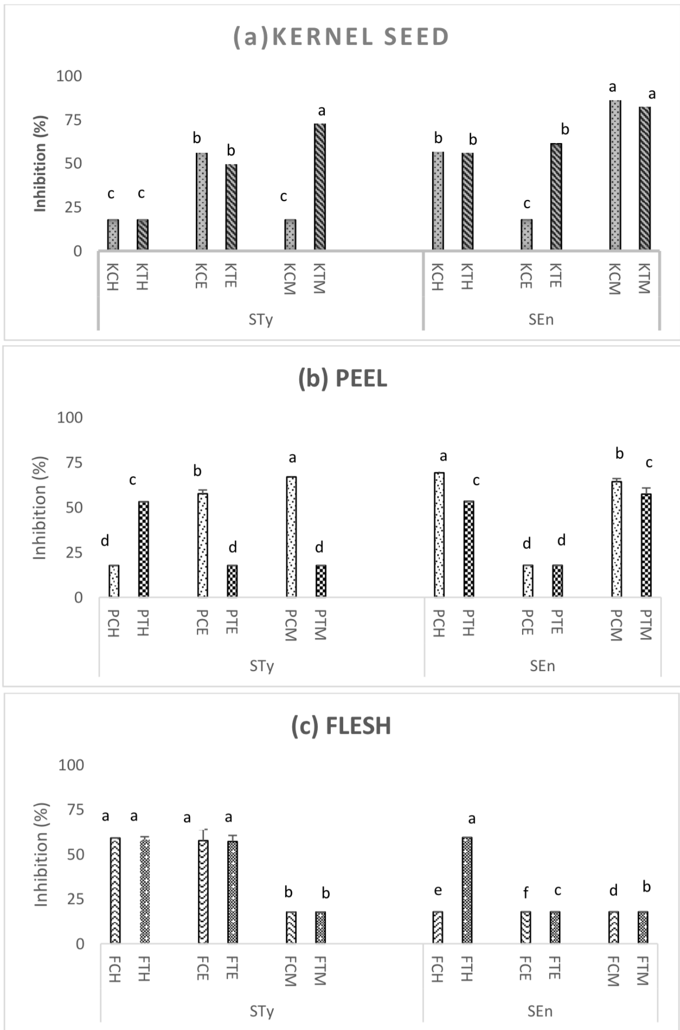

Figure 1. Antipathogenic activity of *M. odorata* kernel seed (a), peel (b) and flesh (c) for control and treated sample against *S. enterica* ser. Typhimurium (STy) and *S. enterica* ser. Enteritidis (SEn). Samples with symbols *H, E* and *M* were respectively referring to the *n*-hexane, ethyl acetate and methanol extracts. The antipathogenic activity was expressed as the percentage of bacterial inhibition (%). Error bars represent the mean ± SD of three replicates. Means that do not share a letter are significantly different (P < 0.05)

compared to *S. enterica* ser. Enteritidis which is more resistant.

3.4 Total phenolic content (TPC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity of Mangifera odorata extracts

3.4.1 Activity of Mangifera odorata kernel seed extracts

The total phenolic content (TPC) of all six *M. odorata* kernel seed extracts were ranged between 0.01–9.72 g GAE/g extract (Table 1). The methanol extracts were rich in TPC whereas most of the hexane and ethyl acetate extracts had a low amount of TPC. Only TPC for (KCM*)* and (KTM) extracts were significantly different ($P < 0.05$) while others were insignificantly different ($P < 0.05$). The ferric reducing antioxidant power (FRAP) values of the six *M. odorata* kernel seed extracts were ranged between 0.05–22.12 g Fe^{2+}/g extract (Table 1). As a whole, all methanol extracts had a higher FRAP value than hexane and ethyl acetate extracts. Similarly, only FRAP values for (KCM) and (KTM) extracts were significantly different ($P<0.05$) while others were insignificantly different ($P<0.05$). The scavenging activity of the *M. odorata* extracts by the inhibition of DPPH was ranged between 18.01 - 89.53% (Table 1). Among the *M. odorata* extracts, the methanol extracts exhibited the highest activity, followed by the ethyl acetate extracts and the lowest activity was exhibited by the hexane extracts. The DPPH scavenging activities between (KCM) and (KTM) extracts however were not significantly different ($P < 0.05$).

Results showed that the *M. odorata* kernel seed extracts possessed the anti-*Salmonellosis* activity against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis. These findings could be correlated with the phenolic content, antioxidant activity and excellent DPPH scavenging activity exhibited by the extracts (Table 1). The methanol extracts of kernel seed were found to be very active and could be attributed to the high amount of TPC, high FRAP values, and high value of scavenging activity. The kernel seed extracts of ethyl acetate were also found as active inhibitors against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis. This can be ascribed to a moderate amount of scavenging activity (44.78-66.06%). However, the activity is somehow uncorrelated to the bioactivity of ethyl acetate extracts of kernel seed as the extracts contained low TPC and FRAP values. Thus, the moderate (45-66%) scavenging activity possibly contributed to the bioactivity.

3.4.2 Activity of Mangifera odorata peel

The TPC of all six *M. odorata* peel extracts were ranged in between 0.18–2.73 g GAE/g extract, while the FRAP values were found in between 0.32–4.36 g Fe^{2+}/g extract (Table 1). The methanol extract (PCM) had a significant amount of TPC, followed by the ethyl acetate

Table 1. Total phenolic content (TPC), ferric reducing antioxidant power (FRAP) and free radical (DPPH) scavenging activity in different parts of *M. odorata* fruit. Samples of *M. odorata* kernel seed, peel and flesh for each control and treated sample were coded with symbols *H, E* and *M* respectively referring to the *n*-hexane, ethyl acetate and methanol extracts. Means that do not share a letter are significantly different ($P < 0.05$)

Sample	Extract		TPC (g GAE/g extract)	FRAP (g Fe^{2+}/g extract)	Scavenging activity (% DPPH inhibition)
Kernel seed	Control	*KCH*	0.024±0.001^{j}	0.067±0.004^{j}	33.99±3.29^{j}
		KCE	0.057±0.001^{j}	0.149±0.002^{j}	66.06±1.67^{f}
		KCM	9.726±0.106^{a}	22.117±0.235^{a}	86.17±1.10^{bc}
	Treated	*KTH*	0.010±0.009^{j}	0.048±0.002^{j}	18.07±0.86^{k}
		KTE	0.038±0.003^{j}	0.087±0.003^{j}	44.78±3.11^{h}
		KTM	8.387±0.050^{b}	17.900±0.054^{b}	89.53±0.05^{ab}
Peel	Control	*PCH*	0.182±0.004^{hi}	0.324±0.013^{i}	52.87±3.91^{g}
		PCE	0.860±0.013^{e}	1.241±0.026^{e}	71.06±3.09^{e}
		PCM	2.728±0.085^{c}	4.366±0.113^{c}	88.87±0.57^{ab}
	Treated	*PTH*	0.236±0.008^{gh}	0.337±0.004^{i}	70.83±0.98^{e}
		PTE	0.951±0.025^{d}	1.270±0.006^{e}	81.01±1.99^{d}
		PTM	0.615±0.007^{e}	1.382±0.029^{d}	89.79±0.20^{ab}
Flesh	Control	*FCH*	0.423±0.003^{f}	0.742±0.017^{g}	38.32±1.21^{i}
		FCE	0.572±0.008^{e}	0.936±0.016^{f}	84.62±5.22^{cd}
		FCM	0.141±0.122^{i}	0.496±0.005^{h}	55.97±2.44^{g}
	Treated	*FTH*	0.293±0.020^{g}	0.584±0.020^{h}	89.41±0.09^{ab}
		FTE	0.302±0.009^{g}	0.609±0.013^{h}	90.94±2.18^{a}
		FTM	0.306±0.008^{g}	0.584±0.021^{h}	89.99±0.33^{ab}

GAE, Gallic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl reagent

(PTE) extract. All methanol extracts had a higher FRAP value than ethyl acetate extracts. The FRAP values for (PCM) and (PTM) extracts were significantly different (P<0.05) whereas between (PCE) and (PTE) were insignificantly different (P<0.05). The hexane extracts for control (PCH) and treated (PTH) peel had low TPC and FRAP values. The scavenging activity from the inhibition of DPPH was ranged between 52.87 – 89.79%. The methanol extracts had a higher scavenging activity than the ethyl acetate extracts. The high inhibition activity of peel extracts could be due to its excellent scavenging activity as demonstrated by the high FRAP values and TPC.

Besides, the *M. odorata* peel extracts also demonstrated anti-*Salmonellosis* activity against *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis (Table 1). The anti-*Salmonellosis* activities of *M. odorata* peel extracts indicated that control peel of methanol extract (PCM) and treated peel of hexane (PTH) extract were found as active inhibitors against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis. On the contrary, (PCH) and (PTM) was found active against *S. enterica* ser. Enteritidis whereas only (PCE) was active against *S. enterica* ser. Typhimurium. The anti-*Salmonellosis* activity of peel extracts against both bacteria could be attributed to the high TPC, FRAP values and scavenging activity in the extracts.

3.4.3 Activity of Mangifera odorata flesh extracts

The TPC and FRAP values in *M. odorata* flesh extracts were ranged from 0.14–0.57 g GAE/g extract and 0.50–0.94 g Fe^{2+}/g extract, respectively (Table 1). As a whole, both TPC and FRAP values quantities were rather low than the peel, which indicated that the flesh extracts contained less amount of TPC and low FRAP values than peel. In comparison, the TPC and FRAP values of control flesh extracts were much higher than that of treated flesh extracts. The TPC amount and FRAP values for all control peel extracts were significantly different (P<0.05) while all treated peel extracts were found insignificantly different (P<0.05). The scavenging activity of all flesh extracts was high (55.97-90.94%) except for FCH (38.32%). The scavenging activity for all control peel extracts were significantly different (P<0.05), however insignificantly difference (P<0.05) was found in the treated peel extracts. The bioactivity of flesh extracts could be attributed to the FRAP value in the flesh extracts.

Table 1 shows that *M. odorata* flesh extracts also possessed moderate anti-*Salmonellosis* activity against *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis. The anti-*Salmonellosis* activities of *M. odorata* flesh extracts indicated that only hexane extract of treated flesh (FTH) was active against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis, while other extracts were only active either against *S. enterica* ser. Typhimurium or *S. enterica* ser. Enteritidis. The anti -*Salmonellosis* activity of flesh extracts against both bacteria was probably due to a moderate amount of TPC, FRAP values and high scavenging activity in the extracts.

4. Discussion

Plant-derived bioactive compounds are usually used to treat diseases. They are partially discovered through established research studies for folk and ethnomedicinal uses (Beutler, 2009). The pharmacological of plant bioactive plays a major part in the pathogenesis due to the resistant strains of bacteria against a drug, which could cause infection and death (Valko *et al*., 2006). Thus, the anti-*Salmonellae* activity, total phenolic content, FRAP value and scavenging activity have revealed that the kernel seed, peel and flesh of *M. odorata* extracts contained bioactivity against *S. enterica* ser. Enteritidis and *S. enterica* ser. Typhimurium. The sequential extraction of kernel seed, peel and flesh of *M. odorata* into three different polarity classes exerted a different level of efficacy towards anti-*Salmonellae* activity. The hexane, ethyl acetate and methanol extracts were represented the nonpolar, medium and polar classes of phytochemicals in the kernel seed, peel and flesh of *M. odorata* extracts, respectively.

Polyphenols (flavonoids, xanthones and phenolic acids), carotenoids, terpenoids and tannins are phytochemicals that found in mango (*M. indica*) and contributed to the main biological property of antioxidant. The major polyphenolic compounds found in mango are mangiferin and quercetin (Nayan *et al*., 2017) while phenolic acids are ascorbic acid and dehydroascorbic acid (Ribeiro *et al*., 2007). Carotenoids are natural organic pigments that gave a bright yellow colour of the peel and flesh of mango (Delgado-Vargas *et al*., 2000). Beta-carotene is the most abundant carotenoids found in mango (Jungalwala and Cama, 1963) and a very good of free radical scavengers (Woodall *et al*., 1997). Terpenoids are volatiles that responsible for aroma in mango (Lalel *et al*., 2003).

The flesh and peel of mango (*M. indica*) contain triterpenes and triterpenoids with lupeol and lupeollinoleate were the most common triterpenoids found in mango (Ruiz-Montanez *et al*., 2014). Tannins contribute to astringent and bitter taste in fruit that affects the nutritional value of the foods by binds to and precipitate protein (Chung *et al*., 1998). Other

eISSN: 2550-2166

phytochemicals found in the flesh and peel of mango are resorcinolic lipids (Engels *et al*., 2009), long-chain fatty acids as such oleic acid, linolenic acid and *n*-pentacosanol; and tocopherols (Ornelas-Paz *et al*., 2007).

The kernel seed of mango contains triterpenes and triterpenoids such as alpha- and beta-pinene, myrcene and limonene, including polyphenols and phenolic acids such as ascorbic acid, mangiferin, quercetin and gallic acid (Anjaneyulu and Radhika, 2000). Other compounds found in mango kernel seed were long-chain hydrocarbons and fatty acids as such stearic acid, eicosanoic acid, linoleic, linolenic, oleic acid, arachidonic acid and palmitic acid including sterols such as stigmasterol, sitosterols and campesterol (Augustin and Ling, 1987). Gallotannins which are hydrolysable tannins is found in the kernel and fruit pulp of mango (Engels *et al*., 2009). Thus, similar phytochemical compounds found in mango (*M. indica*) could possibly found in the *M. odorata* fruit. Those compounds could be responsible for the bioactivity of *M. odorata* that exert significant inhibition of *S. enterica* ser. Enteritidisand *S. enterica* ser. Typhimurium.

Preliminary heat-treatment on *M. odorata* fruit had affected the bioactivity of phytochemicals in the treated extracts compared with the untreated extracts (control). The fruit pretreatment affects the total phenolic content, antioxidant and free radical scavenging activity of the *M. odorata* fruit. Huang *et al*. (2018) indicated that heat treatment and temperature can affect the antioxidant activity of mango peel extract, while Dorta *et al*. (2012) correlated high-temperature treatments with high antioxidant capacity.

Figure 1 shows the antipathogenic activity of (A) kernel seed, (B) peel and (C) flesh for control and treated *M. odorata* extracts. The treated kernel seed extracts (Figure 1A) exert high anti-*Salmonellae* activity against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis between 82.8 to 87.1 of inhibition (%), respectively. The most potent extract was from the control kernel seeds of methanol (KCM) extract with 90.1 of inhibition (%) against the *S. enterica* ser. Enteritidis. Moderate anti-*Salmonellae* activities against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis were exert by the control peel extracts (Figure 1B) between 67.0 to 69.3 %, respectively. The control flesh extracts however only active against *S. enterica* ser. Typhimurium while the treated flesh extracts were found active against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis between 58.1 to 59.4%, respectively.

The inhibition activity of *M. odorata* extracts was analysed by using total phenols assay by Folin-Ciocalteu reagent, ferric reducing antioxidant power (FRAP) and scavenging activity from an electron transfer-based assay. The (ET)-based assays measured the capacity of an antioxidant in reducing an oxidant before a colour change take places (Huang *et al*., 2005). The degree of colour change was correlated with the concentration of antioxidant within the samples. Results show that *M. odorata* extracts had high TPC and FRAP value, which suggested that the phenolic components contributed to both oxidant-reducing and radical scavenging activities of *M. odorata* extracts. The antioxidant activities and TPC values in *M. odorata* are in good agreement with the values reported in the previous study of wild *Mangifera* species (Salahuddin *et al*., 2016).

In this work, the most active extract with the highest antioxidant content was derived from the methanol extract. Methanol was polar solvent thus all of the active compounds including flavonoids in the methanol extracts were polar. On the contrary, hexane extracts were the least active extract, which indicated that the active compounds in the hexane extract were nonpolar. The ET -based assays were found to be more effective on polar extracts than in non-polar extracts, thus rendered a low value in both total phenols and flavonoids contents in the hexane extracts. Acidity has influenced antibacterial activity. The *M. odorata* flesh was acidic hence the extracts were also acidic. Thus, the phenolic groups in flesh extracts were fully protonated and less polar. Besides, the adventitious acidic compounds in the hexane extract may also dramatically influenced the ionization equilibrium of phenols and caused a reduction in inhibition performance (Liu *et al.,* 2019). High TPC (0.42 g GAE/g extract) in hexane extracts (FCH) also suggested that the extract contained an aliphatic-side chain or partially methylated compounds which were easily dissolved in hexane. An example, tocopherols are phenols that are highly soluble in hexane. Such compounds could be present in *M. odorata* flesh (Singh *et al*., 2015) and soluble in the hexane extracts. Thus, these renders the scavenging activity by DPPH assay as a valid assay for antiradical activity of measurement for *M. odorata* extracts.

Bioassay-guided fractionation of *M. odorata* extracts using solvents with increasing polarity order was successful identified potent *M. odorata* extracts with anti -*Salmonellae* activity. The anti-*Salmonellae* activity increased with solvent polarity as shown by treated kernel seed (Figure 1A) against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis*;* and control peel (Figure 1B) against *S. enterica* ser. Typhimurium. The polarity-dependent had increased the total phenolic content (TPC), antioxidant (FRAP) activity and free radical (DPPH) scavenging activity as shown by potent

eISSN: 2550-2166

extracts of (KCM), (KTM) and (PCM) in Figure 1, (A-B). The *M. odorata* methanol potent extracts as such (KCM), (KTM) and (PCM) were high in TPC indicated that the extracts contain strong polar compounds contributed by polyphenols with lower molecular weight. Polar solvent such as methanol is efficient in the extraction of lower molecular weight polyphenols thus used for recovering polyphenols from plant matrices (Do *et al*., 2014). The non-polar *M. odorata* active extracts as such (PTH) and (FTH) contain high free radical (DPPH) scavenging activity indicated that the active phenolic compounds present in those active extracts are non-polar in nature (Nawaz *et al*., 2020). In addition, the differences in the expression of surface proteins and pathogenesis-related protein between bacteria may contribute to the susceptibility of *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis towards the *M. odorata* extracts (Saleh *et al*., 2019). Thus, this work was the first to reveal the anti-Salmonellae activity of six *M. odorata* extracts. The anti-*Salmonellae* activity was correlated to the total phenolics content, antioxidant and scavenging activity in *M. odorata* extracts that effective against both *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis as presented in Table 2.

Table 2. Anti-*Salmonellosis* activity of *M. odorata* extracts. Samples of *M. odorata* kernel seed, peel and flesh for each control and treated sample were coded with symbols *H, E* and *M* respectively referring to the *n*-hexane, ethyl acetate and methanol extracts.

Sample	Extract	*S. enterica* ser. Typhimurium	*S. enterica* ser. Enteritidis
Kernel seed	*KCM*	inactive	potent
	KTM	potent	potent
	KTE	active	moderate
Peel	*PCM*	moderate	moderate
	PTH	active	active
Flesh	*FTH*	active	active

inactive < 50 %; active > 50 – 59 %; moderate > 60-79 % and potent > 80-100 % inhibition

5. Conclusion

To the best of our knowledge, this is the first work that presented the bioactivity of *M. odorata* fruit extracts against *S. enterica* ser. Typhimurium and *S. enterica* ser. Enteritidis. The control kernel seed of methanol extract (KCM) can be served as an active inhibitor against *S. enterica* ser. Enteritidis. Secondly, the treated kernel seed of methanol extract (KTM) exhibited satisfactory inhibition performance against *both S. enteritidis and S. enterica* ser. Typhimurium. Lastly, the control methanol (PCM), treated hexane (PTH) of peel extracts and treated kernel seed of ethyl acetate extract (KTE) demonstrated moderate activity against both *S. enteritidis and S. enterica* ser. Typhimurium. Among all flesh extracts, the treated hexane (FTH) was found to be most active in inhibiting both *S. enteritidis and S. enterica* ser. Typhimurium.

This study has highlighted the importance of *M. odorata* fruits and their potential to be developed into health products for the food and pharmaceutical industries. Thus, the chemical properties using chromatographic and spectroscopic techniques to identify the metabolites in the extracts that responsible for the bioactivities need to be identified in near future. The biological activities focusing on the minimum concentration and dose-dependent manner also essential to be further studied.

Conflict of interest

The authors declare no conflict of interest regarding the publication of this paper.

Acknowledgements

The authors would like to thank the Malaysian Agricultural Research and Development Institute (MARDI) for financial supports in conducting this research funded under Project *PRF407*. Thank you to all of the team members for the technical supports and Mr Saiful Bahri Saari for preparing the treated samples.

References

A'aishah, S. (2014). Keracunan Makanan-Peranan PBT. In Prosiding Persidangan Kesihatan Persekitaran Pihak Berkuasa Tempatan. Putrajaya: Ministry of Urban Wellbeing, Housing and Local Government. [In Bahasa Malaysia].

Adnan, H., Seidel, V. and Tucker, N.P. (2017). Natural antibiofilm agents and the need for antibiofilm drug leads. *Educatum Journal of Science, Mathematics and Technology*, 4(1), 1-8. https://doi.org/10.37134/ejsmt.vol4.1.1.2017

Afshari, A., Baratpour, A., Khanzader, S. and Jamshidi, A. (2018). *Salmonella enteritidis* and *Salmonella typhimurium* identification in poultry carcasses. *Iranian Journal of Microbiology*, 10(1), 45-50

Andino, A. and Hanning, I. (2015). *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. *The Scientific World Journal,* 2015, 520179. https://doi.org/10.1155/2015/520179

Anjaneyulu, V. and Radhika, P. (2000). The triterpenoids and steroids from *Mangifera indica* Linn. *Indian Journal of Chemistry*, 39(12), 883-893

Augustin, M.A. and Ling, E.T. (1987). Composition of mango seed kernel. *Pertanika*, 10(1), 53-59

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Beutler, J.A. (2009). Natural products as a foundation for drug discovery. *Current Protocols in Pharmacology*, 46(1), 9.11.1-9.11.21. https://doi.org/10.1002/0471141755.ph0911s46

Blois, M.S. (1958). Antioxidant determination by the use of a stable free radical. *Nature,* 181, 1533-1535. https://doi.org/10.1038/1811199a0

Brandt, K., Christensen, L. P., Hansen-Moller, J., Hansen, S. L., Haraldsdottir, J., Jespersen, L., Purup, S., Kharazmi, A., Barkholt, V., Frokiaer, H. and Kobaek-Larsen, M. (2004). Health promoting compounds in vegetables and fruits: A systematic approach for identifying plant components with impact on human health. *Trends in Food Science and Technology*, 15(7-8), 384-393. https://doi.org/10.1016/j.tifs.2003.12.003

Chopra, I. and Roberts, M. (2001). Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, 65(2), 232–260. https://doi.org/10.1128/MMBR.65.2.232-260.2001

Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W. and Lin, Y. (1998). Tannins and human health: a review. *Critical Review Food Science and Nutrition*, 38(6), 421-64. https://doi.org/10.1080/10408699891274273

Delgado-Vargas, F., Jimenez, A.R. and Paredes-Lopez, O. (2000). Natural pigments: carotenoids, anthocyanins and betalains – characteristics, biosynthesis, processing and stability. *Critical Reviews in Food Science and Nutrition*, 40(3), 173-289. https://doi.org/10.1080/10408690091189257

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, 22(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001

Dorta, E., Lobo, M.G. and Gonzalez, M. (2012). Reutilization of mango byproducts: study of the effect of extraction solvent and temperature on their antioxidant properties. *Journal of Food Science*, 77 (1), 80-88. https://doi.org/10.1111/j.1750-3841.2011.02477.x

Ediriweera, M.K., Tennekoon, K.M. and Samarakoon, S.R. (2017). A review on ethnopharmacological applications, pharmacological activities and bioactive compounds of *Mangifera indica* (mango). *Evidence-Based Complementary and Alternative Medicine*, 2017, 6949835. https://doi.org/10.1155/2017/6949835

Engels, C., Knodler, M., Zhao, Y.Y., Carle, R., Ganzle, M.G. and Schieber, A. (2009). Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. *Journal of Agricultural and Food Chemistry*, 57(17), 7712-7718. https://doi.org/10.1021/jf901621m

Huang, C.Y., Kuo, C.H., Wu, C.H., Kuan, A.W., Guo, H.R., Lin, Y.H. and Wang, P.K. (2018). Free radical-scavenging, anti-inflammatory, and antibacterial activities of water and ethanol extracts prepared from compressional-puffing pretreated mango (*Mangifera indica* L.) peels. *Journal of Food Quality,* 2018, 1025387. https://doi.org/10.1155/2018/1025387

Huang, D., Ou, B. and Prior, R.L. (2005). The Chemistry behind Antioxidant Capacity Assays. *Journal of Agricultural and Food Chemistry*, 53, 1841−1856. https://doi.org/10.1021/jf030723c

Jungalwala, E.B. and Cama, H.R. (1963). Carotenoids in mango (*Mangifera indica*) fruit. *Indian Journal of Chemistry*, 1(1), 36.

Khandare, M.S. (2016). Mango (*Mangifera indida* Linn) A medicinal and holy plant. *Journal of Medicinal Plants Studies*, 4(4), 44-46.

Lalel, H.J.D., Singh, Z. and Tan, S.C. (2003). Aroma volatiles production during fruit ripening of 'Kensington Pride' mango. *Postharvest Biology and Technology*, 27(3), 323-336. https://doi.org/10.1016/S0925-5214(02)00117-5

Lasano, N.F., Hamid, A., Karim, R., Dek, M.S.P., Shukri, R. and Ramli, N.S. (2019). Nutritional composition, anti-diabetic properties and identification of active compounds using UHPLC-ESI-Orbitrap-MS/MS in *Mangifera odorata* L. peel and seed kernel. *Molecules*, 24(2), 320, https://doi.org/10.3390/molecules24020320

Liu, Q., Tang, G.Y., Zhao, C.N., Gan, R.Y. and Li, H.B. (2019). Antioxidant activities, phenolic profiles, and organic acid contents of fruit vinegars. *Antioxidants*, 8(4), 78. https://doi.org/10.3390/antiox8040078

Ministry of Health (2019). Food poisoning. Retrieved March 31, 2020 from https://www.moh.gov.my.

Mohan, A., Munusamy, C., Tan, Y.C., Muthuvelu, S., Hashim, R., Chien, S.L., Wong, M.K., Kamaruddin, N.A., Podin, Y., Lau, P.S.T., Ng, D.C.E. and Ooi, M.H. (2019). Invasive *Salmonella* infections among children in Bintulu, Sarawak, Malaysian Borneo: a 6-year retrospective review. *BMC Infectious Diseases,* 19, 330. https://doi.org/10.1186/s12879-019-3963-x

Muthu, C., Ayyanar, M., Raja, N. and Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology and Ethnomedicine*, 2, 43. https://doi.org/10.1186/1746-4269-2-43

Nawaz, H., Shad, M.A., Rehman, N., Andaleeb, H. and Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56, e17129. https://doi.org/10.1590/s2175-97902019000417129

Nayan, V., Onteru, S.K. and Singh, D. (2017). Mangifera indica flower extract mediated biogenic green gold nanoparticles: Efficient nanocatalyst for reduction of 4-nitrophenol. *Environmental Progress and Sustainable Energy*, 37(1), 283-294. https://doi.org/10.1002/ep.12669

Ornelas-Paz, J.D., Yahia, E.M. and Gardea-Bejar, A. (2007). Identification and quantification of xanthophyll esters, carotenes and tocopherols in the fruit of seven Mexican mango cultivars by liquid chromatography-atmospheric pressure chemical ionization-time-of-flight mass spectrometry [LC-(APcl+)-MS]. *Journal of Agricultural and Food Chemistry*, 55(16), 6628-6635. https://doi.org/10.1021/jf0706981

Packierisamy, P. R., Raja Haron, R. M. A., Mustafa, M., Ahmad Mahir, H.M., Ayob, A. and Balan, V. (2018). Outbreak caused by food-borne *Salmonella enterica* serovar *Enteriditis* in a residential school in Perak state, Malaysia in April 2016. *International Food Research Journal,* 25(6), 2379-2384.

Ribeiro, S.M.R., De Queiroz, J.H., De Queiroz, M.E.L.R., Campos, F.M. and Sant'Ana, H.M.P. (2007). Antioxidant in mango (*Mangifera indica* L.) pulp. *Plant Foods for Human Nutrition*, 62(1), 13-17. https://doi.org/10.1007/s11130-006-0035-3

Ruiz-Montanez, G., Rogazzo-Sanchez, J.A., Calderon-Santoyo, M., Velazquez-De La Cruz, G. and Navarro-Ocana, A. (2014). Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.). *Food Chemistry*, 159, 267-272. https://doi.org/10.1016/j.foodchem.2014.03.009

Salahuddin, M.A.H., Idris. S. and Mirad, R. (2016). Natural antioxidant properties of selected wild *Mangifera* species in Malaysia. *Journal of Tropical Agriculture and Food Science*, 44(1), 63-72

Saleh, S., Puyvelde, S.V., Staes, A., Timmerman, E., Barbe, B., Jacobs, J., Gevaert, K. and Deborggraeve, S. (2019). *Salmonella Typhi, Paratyphi A, Enteritidis* and *Typhimurium* core proteomes reveal differentially expressed proteins linked to the cell surface and pathogenicity. *PLOS Neglected Tropical Disease*, 13(5), e0007416 https://doi.org/10.1371/journal.pntd.0007416

Singh, R., Singh, K., Maharia, R.S. and Garg, A.N. (2015). Identification of new phytoconstituents and antimicrobial activity in stem bark of *Mangifera indica* (L.). *Journal of Phytochemical and Biomedical Analysis*, 105, 150-155. https://doi.org/10.1016/j.jpba.2014.12.010

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-posphotungustic acid reagent. *American Journal of Enology and Viticulture,* 16, 144-158.

The Sun Daily (2020). Terengganu food poisoning case due to use of expired eggs: Exco. Retrieved from The Sun Daily website: https://www.thesundaily.my/local/terengganu-food-poisoning-case-due-to-use-of-expired-eggs-exco-EY2472723

Valko, M., Rhodes, C.J., Moncol, M., Izakovic, M. and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interaction*, 160(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Woodall, A.A., Lee, S.W.M., Weesie, R.J., Jackson, M. J. and Britton, G. (1997). Oxidation of carotenoids by free radicals: Relationship between structure and reactivity. *Biochemica et Biophysica Acta*, 1336(1), 33-42. https://doi.org/10.1016/S0304-4165(97)00006-8

World Health Organization (2018). *Salmonella* (non-typhoidal). Foodborne disease. *WHO2018*. Retrieved on March 29, 2020 from https://www.who.int/en/news-room/fact-sheets/detail/salmonella-(non-typhoidal).

eISSN: 2550-2166