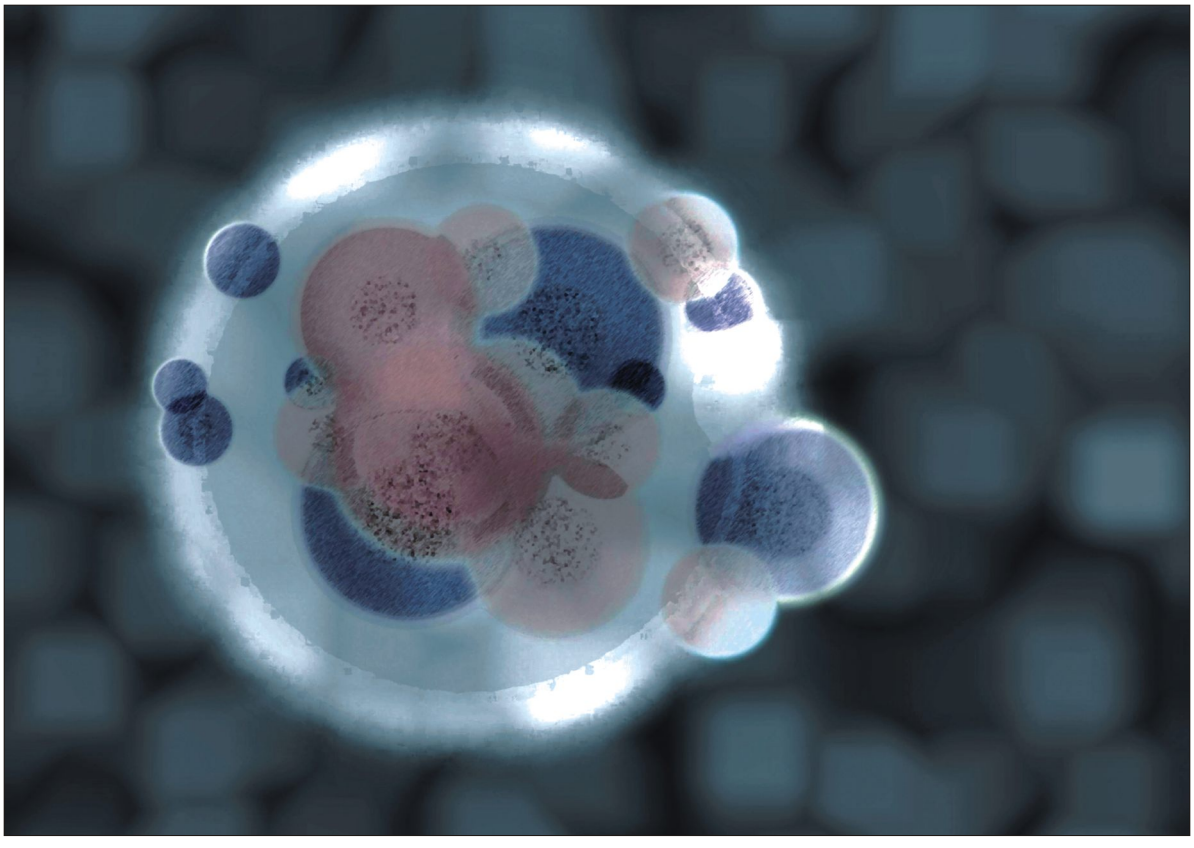

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Research Article
Cytotoxic Activity of Roselle (*Hibiscus sabdariffa* L.) Calyx Extracts against Jurkat T-Lymphoblastic Leukaemia Cells

[1]Normah Awang, [1]Nurul Amalina Abd Aziz, [1]Chan Kok Meng, [1]Nurul Farahana Kamaludin, [2]Rapidah Mohamad and [3]Nor Syaidatul Akmal Mohd Yousof

[1]Programme of Environmental Health and Industrial Safety, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
[2]Programme of Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia
[3]Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Wilayah Persekutuan, Kuala Lumpur, Malaysia

Abstract

Background and Objective: Plant derivative compounds have been widely used in *in vivo* and *in vitro* studies as potential anticancer agents. Several studies demonstrated that Roselle (*Hibiscus sabdariffa* L.) possessed an anticancer effect against few cancer cell lines. However, study on their cytotoxicity against lymphoblastic leukaemia cell lines still remains unclear. Therefore, this study was conducted to assess the cytotoxic effect of *Hibiscus sabdariffa* L. (Roselle) calyx water and ethanol extracts against Jurkat T-lymphoblastic leukaemia cell lines. **Materials and Methods:** A powder of Roselle calyx was extracted using different types of solvent via ultra-sonication extraction method. The extracts obtained were then assessed for their cytotoxicity against Jurkat cells using (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) (MTT) assay upon 24, 48 and 72 h treatment. **Results:** Water solvent gave the highest percentage of yield followed by ethanol solvent, with the least extract came from hexane solvent. The water and ethanol extracts of Roselle calyx gave no IC_{50} values in MTT assay yet demonstrated a decrease in cell viability of Jurkat cells in dose and time-dependant manner. The viability of treated cells at concentrations of 1.0, 0.5, 0.25 and 0.125 mg mL^{-1} of water extract gave a statistical difference ($p<0.05$) compared to untreated cells. Meanwhile, no statistical differences ($p = 0.610$) between cell treated with Roselle ethanol extract at any concentrations with negative control. **Conclusion:** This study indicated that water and ethanol extracts of Roselle calyx were not-toxic towards Jurkat cells. This could be due to the selectivity of phytochemical in which their cytotoxic effect depends on human cancer types. However, those extracts can be further studied for their other potential such as chemo-preventive agent towards Jurkat cell line.

Key words: *Hibiscus sabdariffa* L., Jurkat cells, Roselle calyx extracts, MTT assay, cytotoxic effect

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Corresponding Author: Normah Awang, Programme of Environmental Health and Industrial Safety, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia Tel: +60392897527

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Childhood acute lymphoblastic leukaemia (ALL) is a type of blood cancer in which bone marrow produces too much immature lymphocytes[1] and usually occurred among children aged from 2 to 5 years[2]. Studies found that treatment of ALL could impose side effects on acute lymphoblastic leukaemia patients[3]. Anthracycline used in the treatment of ALL could produce oxygen-free radicals that degraded the cardiac myocytes[4]. Children recovering from ALL were at risk to heart failure, heart attack, cardiac abnormalities and inflammation of the heart epithelium after they received modern chemotherapy treatment[5]. Therefore, various therapeutic treatments which using plants products were introduced in cancer treatment because they were natural, affordable and most of them were non-toxic[6].

Roselle (*Hibiscus sabdariffa* L), a member of Malvaceae family is known as potent antioxidant due to the presence of multiple types of antioxidant and rich with polyphenolic acids, flavonoids and anthocyanins[7] and also one of the potential anticancer agent[8]. The highly valuable part of the plant was their calyxes[9]. Study by Chang *et al.*[10] and Hou *et al.*[11] showed that anthocyanins extracted from the Roselle petals can prevent cell proliferation and caused apoptosis in human HL-60 promyelocytic leukaemia cells[10,11]. The extract also demonstrated anticancer effects in human stomach adenocarcinoma and inhibited cell growth of MCF-7 breast cancer cells[10,12].

Other than cancer treatments, Roselle has also been studied for their protective potential in treating diabetes mellitus (DM). Recent studies were conducted, to evaluate the protective potential of Roselle on the liver and red blood cells (RBC) oxidative stress of streptozotocin-induced diabetic rats[13,14]. Ramalingam *et al.*[15] also reported on the potential of Roselle extract to prevent nicotine-induced cardiac injury and to improve cardiac dysfunction and vasodilation, possibly via modulation of intracellular calcium entry, release and reuptake in the heart[16].

Most of the observed pharmacological effects of Roselle are believed to be mediated through its antioxidant properties[17]. While many studies reported possible beneficial activities of Roselle, little is known about its effects on blood cancer lineage. Hence, this study was conducted to extract Roselle calyx using different type of solvents and investigate their cytotoxic activities against leukaemia cancer cells, Jurkat T-lymphoblastic cell line.

MATERIALS AND METHODS

Plant sample: Roselle (*Hibiscus sabdariffa* L.) calyx powder were purchased from Herbagus Trading Company, Pulau Penang, Malaysia.

Chemicals: Roswell Park Memorial Institute (RPMI) 1640 medium (RPMI) (Sigma-Aldrich, USA), fetal bovine Serum (FBS) (Tico Europe, Netherlands), hydrochloric acid (HCl), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), potassium hydrogen phosphate (KH_2PO_4), potassium chloride (KCl), sodium hydroxide (NaOH) (British Drug House Limited, England), sodium hydrogen phosphate (Na_2HPO_4), sodium bicarbonate ($NaHCO_3$), hexane solvent, dichloromethane solvent, ethanol solvent, antibiotics of penicillin and streptomycin (PAA laboratories GmbH, Austria), dimethyl sulfoxide solution (DMSO) (Merck, Germany), 3-(4.5-dimethylthiazol-2-yl)) -2.5-diphenyltetrazolium bromide (MTT) and 0.4% trypan blue solution (Sigma- Aldrich).

Cell line: Jurkat T-lymphoblastic leukaemia cell lines (Jurkat, Clone E6-1) used in this study was purchased from American Type Culture Collection (ATCC). Dimethyl sulfoxide (DMSO) was used for diluting the roselle extract.

Method: The extraction processed was carried out at the Institute of Medical Research, Kuala Lumpur while the toxicology experiments were done in Bioserasi Laboratory, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia from September, 2016 till May, 2017.

Preparation of *Hibiscus sabdariffa* L. calyx extracts: The procedure used was a slightly modified version of the method described previously[18]. Roselle calyx (*Hibiscus sabdariffa* L.) powders were extracted by using different type of solvents such as water (H_2O), ethanol (EtOH), hexane (Hex) and dichloromethane (DCM) by ultrasonic extraction. The extracts were filtered using Whatman filter paper and then dried using a rotary evaporator (40°C). For water extract, it was dried using freeze drying method. After that, the extracts were kept at -4°C till used. The percentage of yield for each extract was determined based on the formula below:

$$\text{Yield (\%)} = \frac{\text{Final yield obtained (g)}}{\text{Initial sample weight (g)}} \times 100$$

Assessment of cytotoxic activity against Jurkat cells: The cytotoxic effect of Roselle (*Hibiscus sabdariffa* L.) water and ethanol calyx extracts against Jurkat T-lymphoblastic leukaemia cell line was determined using (3-(4,5-dimethylthiazol-2-yl)-2-5 diphenyltetrazolium bromide) (MTT) assay. The procedure used in this study was a modified version of method described by Khaghani *et al.*[19]. Cell viability was measured based on the reduction of tetrazolium salt to the purple formazan by the mitochondrial succinate dehydrogenase enzyme in living cells.

Cell culture: Jurkat T-lymphoblastic leukaemia cell lines were cultured in T-75 culture flask containing RPMI-1640 media supplemented with 10% fetus bovine serum (FBS) and 1% streptomycin. The cells were allowed to grow in an incubator at temperature of 37°C and 5% CO_2.

Preparation of treatment concentration: The water extract was prepared by dissolving it in 1 mL of sterile PBS. For ethanol extract, it was prepared by dissolving 1 g of extract into 1 mL of DMSO. Further serial dilution was conducted to obtain final concentration of 0.1% DMSO. Various concentrations ranging from 0-1 mg mL^{-1} were used to test the cytotoxic effect of Roselle (*Hibiscus sabdariffa* L.) calyx water and ethanol extracts on Jurkat T-lymphoblastic cell lines.

MTT assay: Cells were seeded in a sterile 96-well plate at a density of 1×10^6 cells mL^{-1}. The extracts with various concentrations ranging from 0-1 mg mL^{-1} were added to each well, accordingly and incubated at 37°C in 5% CO_2 for 24, 48 and 72 h. After treatment, 20 µL of MTT salt was added to each well and further incubated for next 4 h. Approximate 180 µL media in each well was removed and replaced with 180 µL of DMSO to dissolve the crystal formazan. After 15 min of incubation, the plate was agitated using orbital shaker for 5 min to ensure the crystal formazan was completely dissolved. The Optical Density (OD) of each well was measured at 570 nm wave length using iMark Microplate Reader (BioRad, USA). The inhibitory concentration that induce cytotoxicity in 50% of cell population (IC_{50}) was calculated and used as a parameter to compare the relative cytotoxicity of each extract. Menadione (MD) was used as a positive control and untreated cells as a negative control.

Statistical analysis: Statistical comparisons were made using one way analysis of variance (ANOVA) to compare the extraction yields of each solvent. One-way ANOVA (*post hoc-dunnett*) test was carried out to determine the cytotoxic effect of Roselle (*Hibiscus Sabdariffa* L.) calyx water and ethanol extracts on Jurkat-T-lymphoblastic leukaemia cell lines by comparing IC_{50} values between treated cells with untreated cells (control). There was significance difference when the value of $p<0.05$.

RESULTS

Extraction of roselle calyx: Figure 1 showed the comparison of extraction yield of Roselle calyx for 4 types of solvents. Water solvent gave the highest percentage of yield compared to other solvents. The order of increasing yield in different solvent extraction system was (Hex and DCM) <EtOH<H_2O. From the ANOVA analysis, the extraction yield of H_2O was significant ($p<0.05$) compared to the others.

Cytotoxic effect of roselle water and ethanol calyx extract against Jurkat cell line: The Table 1 showed the percentage of viability for Jurkat cells, respectively treated with Roselle calyx water and ethanol extracts at different concentration for 24, 48 and 72 h. The percentage of cell viability at 1.0 mg mL^{-1} concentration for the 24, 48 and 72 h treatment period was 81.8±2.4, 89.5±1.9 and 83.5±2.1%, respectively. There was a significant reduction in cell viability of treated cells at concentration of 1.0, 0.5, 0.25 and 0.125 mg mL^{-1} ($p<0.05$) when compared to the untreated cells as negative control. Mean while, the percentage of cell viability at 1.0 mg mL^{-1} of ethanol extract was 79.2±5.9, 89.6±4.8 and 116.8±6.3% for 24, 48 and 72 h treatment period respectively. There was no significant decrease in cell viability of treated cells compared to untreated cells at any concentrations of extract with $p>0.05$. Figure 2 showed a viable cells versus concentration for MD against Jurkat cells and IC_{50} value was obtained at 10.1 µM. There was a significant

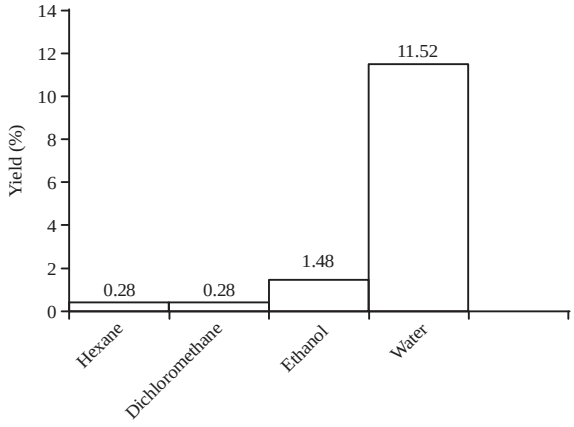

Fig. 1: Yield (%) of roselle (*Hibiscus sabdariffa* L.) calyx extracts

Table 1: Cell viability (%) treated with Roselle calyx water and ethanol extract for 24, 48 and 72 h

	Viability (%)					
	Water extract			Ethanol extract		
Concentration (mg mL^{-1})	24 h	48 h	72 h	24 h	48 h	72 h
1.0	81.8±2.4*	89.5±1.9*	83.5±2.1*	79.2±5.9	89.6±4.8	116.8±6.3
0.5	82.1±2.3*	89.8±3.3*	89.1±4.1*	80.5±6.6	91.2±6.3	108.5±4.8
0.25	90.7±5.2*	93.4±4.9*	86.0±2.7*	83.4±6.3	91.0±1.6	99.8±6.5
0.125	83.1±8.0*	91.6±0.8*	87.8±2.3*	83.3±3.6	94.2±3.1	95.6±3.0
0.0625	97.5±2.7	94.7±6.2	87.8±4	86.5±4.5	92.6±1.1	98.1±3.3
0	100	100	100	100	100	100

Data represent mean value of three replicates±SEM, Positive control: Menadione, negative control: Untreated cells, *The significant difference ($p<0.05$) compared to the negative control

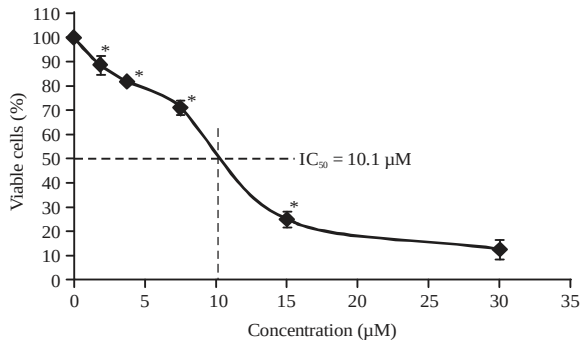

Fig. 2: Cytotoxic effect on Jurkat cells treated with menadione for 24 h

Data represent Mean±SEM of three replicates. *Significant difference ($p<0.05$) compared to untreated cells

difference ($p<0.05$) in percentage of viable cells when compared to untreated cells for all concentrations of MD. Based on the viability of cells, when treated with Roselle calyx extracts and MD, it showed that Roselle calyx extracts were not-toxic towards Jurkat cells compared to MD.

DISCUSSION

In the present research calyx extract of Roselle (*Hybiscus sabdariffa* L.) was successively extracted using ultra-sonication method with different type of solvents such as water, ethanol, hexane and dichloromethane.

The order of increasing yield in different solvent extraction system was (DCM and Hex) <EtOH<H_2O. Each of solvents gave different yield (%) due to their different polarity index whereas water has the highest polarity index which was 9.0 followed by ethanol (5.2), dichloromethane (3.7) and hexane (0)[20].

The results suggested that the major phytochemicals in Roselle calyx were mostly high in polarity and soluble in water. It was supported by the previous study that Roselle calyx was found to contain high anthocyanin, the largest water soluble pigments in plant[21]. This observation indicated that polar compounds were easier to be extracted compared to non-polar compounds. Although both water and ethanol contain hydroxyl group that can form hydrogen bonding with the solute, water is more effective in extracting the solute because it has higher polarity and shorter chain[22]. These characteristics of water improved its capability to extract the polar compounds. Thus, it explained the significant observed between water and ethanol. The difference in yields for other solvents may be due to other factors including phytochemicals in plants, extraction temperature, extraction time and solvent to solid ratio[23].

The cytotoxicity test in measuring the cell growth or cell death has been widely used to identify potential anticancer agents[24]. In this study, water and ethanol extract of Roselle calyx were tested for their cytotoxicity against Jurkat cells, a T-lymphoblastic leukaemia cells. Upon 24 h treatment, both extracts showed no cytotoxic effects on Jurkat cells as no IC_{50} was obtained. Thus, we increased the treatment time up to 72 h for both extracts. In case of Roselle calyx water extract, at highest concentration of 1 mg mL^{-1}, the cell viability increased from 24-48 h, then decreased from 48-72 h treatment. Meanwhile, when treated with ethanol extract, the viability of Jurkat cells at concentration of 1 mg mL^{-1} increased in time-dependant manner. The scenario could be related to the adaptive response of the cells towards the duration of treatment[25]. In this study, menadione which was used as a positive control was also able to produce cytotoxic effect against Jurkat cells and gave IC_{50} at 10.1 μM and the data was in good agreement with the previous report[26].

Anthocyanins and polyphenols like protocatechuic acid and quercetin are amongst the most important phytochemicals that described the physiological activity in *Hibiscus sabdariffa* L. However, anticancer activity of polyphenols towards blood cancer cells depends on the origin of the cells either myeloid, lymphoid or erythroid[27]. Quercetin

which is normally available in the Roselle calyx has shown higher cytotoxic activity against myeloid cancer cells compared to lymphocytic cancer cells[27]. Thus, it could explain the disparate findings obtained in current study as Roselle calyx showed no cytotoxicity towards Jurkat T-lymphoblastic leukaemia cells.

Besides, Roselle calyx is rich in antioxidant called flavonoid. This antioxidant protected the cells by scavenging the reactive oxygen species[28].Pervious study also found that Roselle treatment at concentration of 50 and 100 μg mL^{-1} showed a protective effect on LPS-induced microglial C8-B4 cells[29]. In cancer treatment, some chemotherapeutic agents generated free radicals in order to induce cellular damage and necrosis of malignant cells. However, this antioxidant was found to protect not only the healthy cells but also tumor cells from oxidative damage caused by the chemotherapeutic agents[30-32]. These findings indicated that Roselle calyx extract could also protected the Jurkat T-lymphoblastic leukaemia cells from cell death.

This study implied the cytotoxicity knowledge of Roselle calyx extract towards acute lymphocytic leukaemia. Even though the extracts have not shown any cytotoxic activity against Jurkat T-lymphoblastic cell lines, it was expected to show some other potential activity such as therapeutic agent. Thus, further study should be conducted to explore in depth about the other potential of extract of Roselle calyx.

CONCLUSION

Water gave the highest extraction yield, indicated that phytochemical found in the Roselle calyx were mostly polar compounds. MTT assay results showed that Roselle calyx extracts exerted no cytotoxicity towards Jurkat leukaemia cells. However, this study provided only basic data, further studies are necessary for isolation and identification of biologically active substances from these extracts which might be responsible for other therapeutic activity.

SIGNIFICANCE STATEMENT

This study discovered that Roselle calyx contained mostly polar phytochemical compounds in which the information was important to describe the potential of this extract in other biological activities. The cytotoxicity screening found that this extract showed no toxicity towards acute lymphocytic leukaemia, Jurkat cell line. However, this finding was significant as a fundamental study to assess the potential of Roselle calyx extract in acute lymphoblastic leukaemia. This study will facilitate the future researches to disclose the cytotoxic activity of Roselle calyx extract in different blood lineage and also discover other potential of this extract as therapeutic agent.

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REFERENCES

1. National Cancer Institute, 2017. Childhood acute lymphoblastic leukemia treatment (PDQ®)-health professional version. National Cancer Institute. https://www.cancer.gov/types/leukemia/hp/child-all-treatment-pdq.
2. Inaba, H., M. Greaves and C.G. Mullighan, 2013. Acute lymphoblastic leukaemia. Lancet, 381: 1943-1955.
3. Huang, T.T. and K.K. Ness, 2011. Exercise interventions in children with cancer: A review. Int. J. Pediatr., Vol. 2011. 10.1155/2011/461512
4. Adams, M.J. and S.E. Lipshultz, 2005. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: Implications for screening and prevention. Pediatr. Blood Cancer, 44: 600-606.
5. Printz, C., 2010. Adult survivors of childhood and adolescent cancer have more heart disease. Cancer, 116: 2507-2507.
6. Roja, G. and P.S. Rao, 2000. Anticancer compounds from tissue cultures of medicinal plants. J. Herbs, Spices Med. Plants, 7: 71-102.
7. Ochani, P.C. and P. D'Mello, 2009. Antioxidant and antihyperlipidemic activity of *Hibiscus sabdariffa* Linn. leaves and calyces extracts in rats. Indian J. Exp. Biol., 47: 276-282.
8. Ali, B.H., N. Al Wabel and G. Blunden, 2005. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. Phytother. Res., 19: 369-375.
9. Da-Costa-Rocha, I., B. Bonnlaender, H. Sievers, I. Pischel and M. Heinrich, 2014. *Hibiscus sabdariffa* L.-a phytochemical and pharmacological review. Food Chem., 165: 424-443.
10. Chang, Y.C., H.P. Huang, J.D. Hsu, S.F. Yang and C.J. Wang, 2005. Hibiscus anthocyanins rich extract-induced apoptotic cell death in human promyelocytic leukemia cells. Toxicol. Applied Pharmacol., 205: 201-212.
11. Hou, D.X., X. Tong, N. Terahara, D. Luo and M. Fujii, 2005. Delphinidin 3-sambubioside, a *Hibiscus* anthocyanin, induces apoptosis in human leukemia cells through reactive oxygen species-mediated mitochondrial pathway. Arch. Biochem. Biophys., 440: 101-109.

12. Lin, H.H., J.H. Chen, W.H. Kuo and C.J. Wang, 2007. Chemopreventive properties of *Hibiscus sabdariffa* L. on human gastric carcinoma cells through apoptosis induction and JNK/p38 MAPK signaling activation. Chemico-Biol. Interact., 165: 59-75.
13. Nafizah, A.H.N., S.B. Budin, A.H. Zaryantey, A.R. Mariati and R.L. Santhana *et al*., 2017. Aqueous calyxes extract of roselle or *Hibiscus sabdariffa Linn* supplementation improves liver morphology in streptozotocin induced diabetic rats. Arab J. Gastroenterol., 18: 13-20.
14. Mohamed, J., S.W. Shing, M.H.M. Idris, S.B. Budin and S. Zainalabidin, 2013. The protective effect of aqueous extracts of roselle (*Hibiscus sabdariffa* L. UKMR-2) against red blood cell membrane oxidative stress in rats with streptozotocin-induced diabetes. Clinics, 68: 1358-1363.
15. Ramalingam, A., S.B. Budin, Y.C. Lim, Y.N.L. Si and S. Zainalabidin, 2016. Dietary UKMR-1 roselle supplementation prevents nicotine-induced cardiac injury by inhibiting myocardial oxidative stress. Sains Malaysiana, 45: 1131-1137.
16. Si, L.Y.N., S.A.M. Ali, J. Latip, N.M. Fauzi, S.B. Budin and S. Zainalabidin, 2017. Roselle is cardioprotective in diet-induced obesity rat model with myocardial infarction. Life Sci., 191: 157-165.
17. Abdul Hamid, Z., W.H.L. Lin, B.J. Abdalla, O.B. Yuen and E.S. Latif *et al*., 2014. The role of *Hibiscus sabdariffa* L. (Roselle) in maintenance of *Ex Vivo* murine bone Marrow-derived hematopoietic stem cells. Scient. World J., 10.1155/2014/258192
18. Annegowda, H.V., R. Bhat, L. Min-Tze, A.A. Karim and S.M. Mansor, 2012. Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. J. Food Sci. Technol., 49: 510-514.
19. Khaghani, S., F. Razi, M.M. Yajloo, M. Paknejad, A. Shariftabrizi and P. Pasalar, 2011. Selective cytotoxicity and apoptogenic activity of *Hibiscus sabdariffa* aqueous extract against MCF-7 human breast cancer cell line. J. Cancer Ther., 2: 394-400.
20. Snyder, L.R., 1974. Classification of the solvent properties of common liquids. J. Chromatogr. A, 92: 223-230.
21. Khalaphallah, R. and W.S. Soliman, 2014. Effect of henna and roselle extracts on pathogenic bacteria. Asian Pac. J. Trop. Dis., 4: 292-296.
22. Pin, K.Y., A.L. Chuah, A. Abdull Rashih, M.P. Mazura, J. Fadzureena, S. Vimala and M.A. Rasadah, 2010. Antioxidant and anti-inflammatory activities of extracts of betel leaves (*Piper betle*) from solvents with different polarities. J. Trop. For. Sci., 22: 448-455.
23. Abdul Razak, M.F.B., P.K. Yong, ZM. Shah, L.C. Abdullah, S.S. Yee and I.T.C.S. Yaw, 2012. The effects of varying solvent polarity on extraction yield of *Orthosiphon stamineus* leaves. J. Applied Sci., 12: 1207-1210.
24. Liu, T.Z., J.T. Cheng, S.J. Yiin, C.Y. Chen, C.H. Chen, M.J. Wu and C.L. Chern, 2008. Isoobtusilactone A induces both caspase-dependent and -independent apoptosis in Hep G2 cells. Food Chem. Toxicol., 46: 321-327.
25. Crawford, D.R. and K.J. Davies, 1994. Adaptive response and oxidative stress. Environ. Health Perspect., 102: 25-28.
26. Bonilla-Porras, A.R., M. Jimenez-Del-Rio and C. Velez-Pardo, 2011. Vitamin K3 and vitamin C alone or in combination induced apoptosis in leukemia cells by a similar oxidative stress signalling mechanism. Cancer Cell Int., Vol. 11. 10.1186/1475-2867-11-19
27. Sak, K., 2014. Cytotoxicity of dietary flavonoids on different human cancer types. Pharmacogn. Rev., 8: 122-146.
28. Mohd-Esa, N., F.S. Hern, A. Ismail and C.L. Yee, 2010. Antioxidant activity in different parts of roselle (*Hibiscus sabdariffa* L.) extracts and potential exploitation of the seeds. Food Chem., 122: 1055-1060.
29. Rajab, N.F., S.M. Musa, M.A. Munawar, L.L. Mun, H.K. Yen, F.W. Ibrahim and C.K. Meng, 2016. Anti-neuroinflammatory effects of *Hibiscus sabdariffa* Linn. (Roselle) on lipopolysaccharides-induced microglia and neuroblastoma cells. Malaysian J. Health Sci., 14: 111-117.
30. D'Andrea, G.M., 2005. Use of antioxidants during chemotherapy and radiotherapy should be avoided. CA: Cancer J. Clinicians, 55: 319-321.
31. Lawenda, B.D., K.M., Kelly, E.J. Ladas, S.M. Sagar, A. Vickers and J.B. Blumberg, 2008. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J. Natl. Cancer Inst., 100: 773-783.
32. Salganik, R.I., 2001. The benefits and hazards of antioxidants: Controlling apoptosis and other protective mechanisms in cancer patients and the human population. J. Am. Coll. Nutr., 20: 464-472.