MJFAS MALAYSIAN JOURNAL OF FUNDAMENTAL AND APPLIED SCIENCES
PRINT ISSN: 2289-5981 | ONLINE ISSN: 2289-599X

RESEARCH ARTICLE

Effect of extraction solvents on the phytochemical content and bioactivity of *Momordica charantia* Linn. fruits

Nurul Dalila Abdul Rahim [a], Harisun Yaakob [a], Rosnani Hasham @ Hisam [a], Mohamad Roji Sarmidi [b], Kian-Kai Cheng [b,*]

[a] *Department of Bioprocess and Polymer Engineering, School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Skudai 81310, Johor, Malaysia*
[b] *Innovation Centre in Agritechnology, Universiti Teknologi Malaysia, 84600 Muar, Johor, Malaysia*

* *Corresponding author: chengkiankai@cheme.utm.my*

Article history
Received 15 June 2020
Revised 9 September 2020
Accepted 22 October 2020

Abstract

Momordica charantia (*M. charantia*) is a herbaceous climber commonly found in Southeast Asia with therapeutic importance for various illnesses. This study focused on the effect of extraction solvents on saponins-containing compounds from *M. charantia* and their bioactivities. Different organic solvents including water, ethanol, ethyl acetate, a mixture of methanol-water and methanol-n-butanol were used in the extraction process. The total saponin content, total flavonoid and phenolic content for each extract were examined. In addition, the antioxidant capacity of these extracts were evaluated using both 1,1-diphenyl-2-picrylhydrazyl (DPPH) Free Radical Scavenging Activity and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay. Furthermore, α-amylase and lipase inhibition assay were carried out using an in vitro model. The result showed that methanol-n-butanol extracts exhibited the highest total saponin, flavonoid, phenolic content, and ABTS antioxidant activity compared to the other extracts. The α-amylase inhibition assay revealed that water extract and methanol-n-butanol extract from *M. charantia* contained potent α-amylase inhibitor. On the other hand, the ethyl acetate extract was found to have the most antioxidant capacities based on DPPH radical scavenging assay. The ethyl acetate extract also exhibited the highest inhibition of lipase activities. In conclusion, the methanol-n-butanol solvent was found to be the most effective in extracting saponin from *M. charantia*. The *M. charantia* extracts showed inhibition of α-amylase and lipase activities which may suggest the therapeutic potential of *M. charantia* for obesity and diabetes.

***Keywords*:** Bioactivity, *Momordica charantia*, phytochemical, saponin-containing compounds.

INTRODUCTION

Obesity is a complex multifactorial disease which correlates closely with type 2 diabetes and cardiovascular diseases. The incidence of obesity in the world has increased by more than two-fold since 1980 according to World Health Organization. It has reached epidemic proportions and is a major contributor to the global burden of chronic diseases and disabilities with incalculable social costs. (Yun, 2010). For the past 30 years, Malaysia has become the "fattest" country in Asia, with almost half the adult population now overweight or obese (https://umsc.my/?umsc_news=malaysia-has-become-fattest-country-in-asia-in-30-years, 15 May 2020). Anti obesity drugs such as orlistat and sibutramine may lead to side effects including hypertension, insomnia, constipation, headache, and dry mouth (Yun, 2010). Therefore, there are increasing research interest on bioactive ingredient from natural product as an alternative natural anti-obesity agent.

Bitter gourd (*Momordica charantia*) claimed to have therapeutic capacities for the treatment of obesity and metabolic disorders. Various saponins in bitter gourd, both steroids and triterpenoids, in their flesh and seeds (Tan *et al*., 2016) are used as antihyperglycemic herbal medicine to manage obesity and diabetes complications. Although saponins from this fruit have considerable potential for anti-obesity, there are limited studies for efficient processing and utilization of *M. charantia*'s saponin for anti-obesity treatment. Saponins are an amphipathic molecules which has both polar and non-polar parts. Thus a mixture of polar and non-polar solvent of choices are necessary for effective saponin extraction. These secondary metabolites were found to inhibit pancreatic lipase. They have been extracted using ethanol, methanol, n-butanol and ethyl acetate as solvents (Shobha *et al*., 2017). Previously, V. Le *et al*., (2018 b) indicated that the highest content of saponins was achieved from using water-saturated n-butanol and methanol extracts. Meanwhile, Barve & Jayakumar (2010) suggested to use methanol for separation or isolation of saponin constituents.

The aim of the current studies was to identify the most effective solvent for saponin extraction from small bitter gourd using five different solvents. The obtained crude extracts were subjected to phytochemical analysis including the DPPH radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) antioxidant assay, total saponin, flavonoid, phenolic content and an in-vitro model such as lipase inhibition and α-amylase inhibition.

MATERIAL AND METHOD

Sample preparation

Fresh *M. charantia* fruits were bought from a local Malaysian market, a voucher specimen (UNMC5600/19) deposited in the Herbarium, School of Pharmacy, Faculty of Science, University of Nottingham Malaysia. The fruits were washed thoroughly with distilled water, cut into 2 cm slices and stored at 20 ^{0}C overnight. Washed fresh fruits were freeze-dried using Cuddon FD 80, New Zealand. Lyophilized whole fruits were subsequently grounded into fine powder using Panasonic High Power Grinder, were sieve into 1 mm particle size and stored in a - 20 ^{0}C freezer for solvent extraction.

Solvent extraction

All of the fruit samples (6 g each) were subjected to successive solvent extraction with five different solvents. Sample was extracted in both water and 95 % of ethanol for 15 m at 40 ^{0}C (1:20, g/ml) namely Extract A & B, respectively, adopted and modified from Tan *et al*., (2014 a). Sample was extracted in ethyl acetate for 48 h in the dark at room temperature (1:15, g/ml) (Extract C), while pellet was further extracted in methanol for 2 h in the dark at room temperature (1:15, g/ml). The supernatant was dried and later dissolved in water-n-buthanol, to produce methanol-water extract (Extract D), and methanol-n-butanol extract (Extract E). All extraction were done by maceration. Extracts were then filtered through vacuum filter with Whatman No. 4 filter paper, evaporated at 40 ^{0}C oven and stored at 20 ^{0}C for further analysis.

Total Saponin Contents

Based on a modified method of Perez *et al*., (2019) and V. Le *et al*., (2018 a), the Total Saponin Content was quantified using the vanillin-sulphuric acid assay. 500 µl sample or standard Escin (1 mg/ml) was mixed with 500 µl vanillin in a glass vial. Then, 5 ml of 72 % H_2SO_4 was added using glass pipette to the glass vial placed on ice bath. The vials were heated at 60 ^{0}C water bath for 15 minutes and then cooled in ice bath for another 15 minutes. The absorbance were measured at 544 nm with UV-Vis spectrophotometer.

Total Phenolic Contents

The Total Phenolic Content were determined according to the Folin-Ciocalteau (FCR) procedure (Akanni *et al*., 2014). Briefly, 20 µl sample or standard Gallic acid was mixed with 100 µl 0.2N FCR. Reaction mix were incubated for 5 minutes. Later, 80 µl 7.5 % Sodium carbonate (Na_2CO_3) was added and mixture was incubated for an hour. Absorbance were read at 750 nm wavelength using Elisa microplate reader. Gallic acid (20-100 µg/ml) were used as standard chemical for calibration. Results were expressed as mg GAE/ g.

Total Flavonoid Contents

The Total Flavonoid Content was valuated using the method of Akanni and colleague (2014). Briefly, 0.3 ml sample or standard Rutin was mixed with 0.3 ml reagent 5 % Sodium nitrite ($NaNO_2$) and preincubated at room temperature for 5 minutes. 0.3 ml 10 % Aluminium chloride ($AlCl_3$) was added and mixture was incubated for 6 minutes. 2 ml 1 mol/L Sodium hydroxide (NaOH) and 7.1 ml distilled water were then added and vortexed. Absorbance were read at 510 nm wavelength on Shimadzu 1800 UV-Vis spectrophotometer. Rutin (0.2-1 mg/ml) were used as standard chemical for calibration. Results were expressed in mg RE/ g.

Antioxidant activity

Both the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging as well as 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay were used to quantify the antioxidant activity of *M. charantia* extracts according to method by Akanni *et al*., (2014).

DPPH Free Radical Scavenging Activity

For the DPPH assay, 1 ml of sample extracts was mixed with 1 ml of 0.005 M DPPH in MeOH and incubated in the dark for 30 min. The absorbance was measured at 517 nm with UV-Vis spectrophotometer. The scavenging activity was calculated as follows:

$$[(Ao - Ae)/Ao] \times 100\ \% \quad (1)$$

(Ao = absorbance without extract; Ae = absorbance with extract).

ABTS Assay

For the ABTS assay, 1 ml sample extract was mixed with 3 ml of ABTS reagents, vortexed and incubated for 10 minutes in the dark. The absorbance was measured at 734 nm with UV-Vis spectrophotometer. The ABTS+ scavenge % was calculated as:

$$ABTS\ scavenge\ \% = [(Ao - Ae)/Ao] \times 100\ \% \quad (2)$$

(Ao = absorbance without extract; Ae = absorbance with extract).

Lipase Inhibitory Activity

Pancreatic lipase inhibition experiments of *M. charantia* extracts was held based on the method described by Yaakob., H (2010). In this experiment, the effect of bile salts and Phosphatidylcholine (PC) during lipid digestion of sample was determined by measuring a free fatty acid release. The lipolysis experiments were done using a Metrohm autotitrator (Switzerland). Briefly, 1 mL of samples was added to 99 mL of 2 mM Bis-tris buffer containing 0.15 M Sodium Chloride (NaCl) and 0.01 M Calcium Chloride ($CaCl_2$). 15 mL sample was transferred into a reaction vessel with the temperature regulated at 37 °C. PC and bile salts were added into the mixture to reach final concentrations of 0.42 mM and 9.7 mM, respectively. The reaction mixture was stirred with the pH regulated to 7 with addition of 0.01 M Sodium Hydroxide (NaOH) and 0.1 N Hydrochloric acid (HCl). Lipolysis was started by adding 10 nM co-lipase and 2 nM pancreatic lipase into the reaction mixture and the pH was maintained at pH 7 by autotitrating 0.01 M NaOH into the mixture. The experiments were repeated in the absence of PC and bile salts. The volume of NaOH consumed in the lipolysis experiments was corrected by subtracting the amount consumed when the experiment was run with the buffer solution alone and represent as free fatty acid release.

α-amylase Inhibitory Activity

The assay was conducted using the chromogenic method adopted and modified from Sigma–Aldrich. 250 µl sample extract different concentration was mixed with 250 µl α-amylase solution, vortexed and incubated for 10 minutes. 250 µl starch solution was added and further incubated for 10 minutes. 500 µl DNSA solution was added to the reaction mixture and incubated in 80 ^{0}C water bath for 10 minutes, and then cooled to room temperature. 5 ml distilled water was added before absorbance measured at 540 nm using UV-Vis spectrophotometer. The assay was performed in triplicates and α-amylase inhibitory activity was expressed as percent inhibition and calculated as:

$$\alpha\text{-}Amylase\ inhibitory\ activity\ (\%) = (1 - OD\ test\ sample/OD\ control) \times 100 \quad (3)$$

(OD = optical density)

Statistical Analysis

All data were expressed as mean ± SD (n = 3). Statistical analysis of the results was performed using one-way ANOVA, followed by

Tukey's multiple comparison post hoc tests to determine the differences among organic solvents and phytoconstituents tests, with significant differences observed at $p < 0.05$ using the IBM SPSS statistical software version 23.0.

RESULTS AND DISCUSSION

Effect of solvent on the phytoconstituents

The differences in the polarity of each solvent will extract different metabolites. Polar solvents such as water will typically extract polar metabolites and non-polar solvent will dissolve less polar metabolites. From the analysis, it was found that the methanol-n-butanol extract (extract E) showed the highest concentrations of secondary metabolites such as saponins, phenolic and flavonoid. A serial extraction with methanol and n-butanol solvent yielded the maximum saponin content amounted 5414.19 ± 101.62 mg EE/g, highest flavonoid content (1612.55 ± 59.89 mg RE/g) and most phenolic content (31.72 ± 1.05 mg GAE/g) compared to the other four extracts. These screening of phytochemicals were done by using colorimetric method showed the most TSC in extract E followed by methanol-n-butanol extract (C), methanol-water extract (D), water extract (A) and ethanol extract (B). On the other hand, the TFC was found most abundant in extract E, then C, D, B and A. The highest phenolic content was observed in the methanol-n-butanol extract, followed by extracts C, A, D and B. This finding is consistent with reports by Srinivasulu *et al*., (2017) which 74.65 ± 0.04 GAE mg/g phenolic content were identified from methanol extract followed by ethyl acetate (62.16 ± 0.07 GAE mg/gm).

For the antioxidant capacity, methanol-n-butanol extract (E) has the highest in the ABTS assay and second highest in DPPH assay. On contrary, water extract (A) exhibit the lowest ABTS and DPPH values. Five extracts prepared with different solvent were tested on an in-vitro model namely α-amylase and lipase inhibitory activities. The inhibitory activity of α-amylase which related directly to the metabolism of glucose, and lipase which related directly to the metabolism of fatty acids were tested to evaluate their hypoglycemic activity. These tests revealed that the methanol-n-butanol extract was most potent α-amylase inhibitor and third potent lipase inhibitor of after ethyl acetate and methanol-water extracts. The differences in metabolites extracted were possibly caused by the differences in the polarity of each solvent. Polar solvents such as water will typically extract polar metabolites and non-polar solvent will dissolve less polar metabolites.

Total Saponin Content

The total Saponin content (TSC) of all extracts ranged from 3870.69–5414.19 mg EE/ g DW. Extract E (Methanol-n-butanol) yielded the most saponins while extract B (Ethanol) yielded the least saponins. This is consistent with findings by V. Le *et al*., (2018 b) which reported highest TSC in the methanol and butanol extracts which was not significantly different from each other which are 41 mg AE/g and 40 mg AE/ g respectively. Furthermore, Barve *et al*., (2010) also found methanol was the most effective to extract saponin and Ngo *et al*., (2017) who found methanol was the second most effective. On the contrary, Tan *et al*., (2014 a) reported more saponin composition in ethanol extract compare to aqueous extract possibly because the study was optimized using response surface methodology.

Total Phenolic Content

The total phenolic contents (TPC) of all extracts ranged from 13.79–31.72 mg GAE/g DW. The methanol-n-butanol solvent had the highest TPC followed by ethyl acetate, water, methanol-water and ethanol (figure 3) as opposed to a reports by Tan *et al*., (2014 c) which found maximum amount of TPC in ethanol extract and minimum amount in n-butanol extract. Previously, Akter *et al*., (2019) reported the variable solubility of polyphenols in different solvents and the cellular macromolecules's complex structure within different plant tissues are possibly causing varieties of TPC obtained in different extracts.

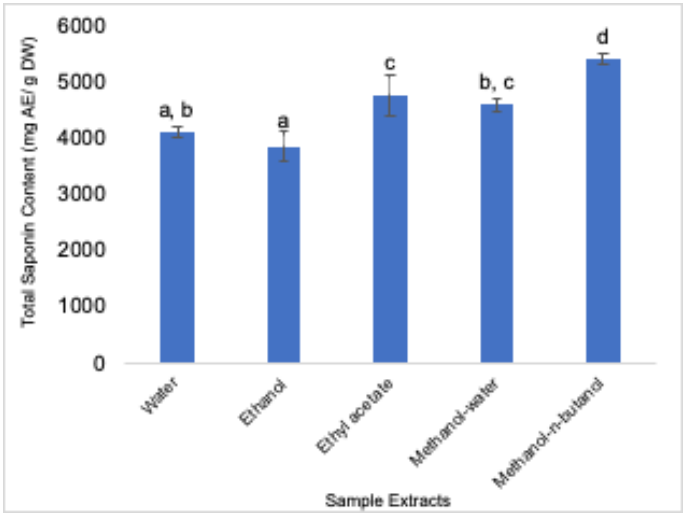

Fig. 1 Total saponin contents of five *M. charantia* extracts. Results are expressed as mean ± SD; (n = 3). Mean values of each column with different superscript letters (a, b, c, d) are significantly different at $p < 0.05$. AE: Aescin equivalents.

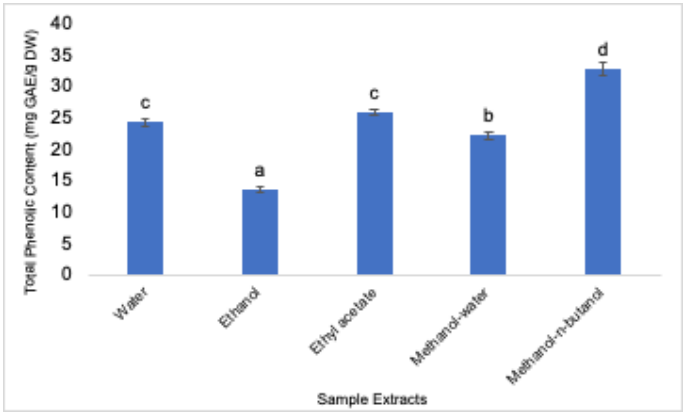

Fig. 2 Total phenolic contents of five *M. charantia* extracts. Results were expressed as mean ± SD; (n = 3). Columns with different superscript letters (a, b, c, d) are significantly different from each other ($p < 0.05$). GAE: Gallic acid equivalents.

Total Flavonoid Content

The results demonstrate that at the concentration of 0.5 mg/ml, the total flavonoid contents (TFC) for all extracts ranged from 73.73–1612.55 mg RE/g DW with the highest amount of TFC obtained from methanol-n-butanol extract and the lowest amount was obtained from water extract. Our findings were in agreement with previous studies by Tan *et al*., (2014 a) which suggested more TFC was found from ethanol extract compared to water and Tan *et al*., (2014 b) which found that TFC least dissolved in polar solvents such as water. However a study by Ngo *et al*., (2017) recorded 50 % Acetone extract yielded highest TFC whereas absolute acetone extract yielded lowest TFC with contradicting results.

Effect of solvent on the antioxidant activities and enzymes inhibitory activities

DPPH Free Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used to determine the antioxidant activity of MC extracts. At the concentration of 1 mg/ml, ethyl acetate extract exhibited the highest DPPH radical scavenging activity. Methanol-n-butanol and ethanol extracts were also had the most antioxidant capacity but methanol-water and water extracts had significantly lower antioxidant capacity as supported by

Tan *et al*., (2014 a). The differences of antioxidant activity for each solvents might be due to the variations of bioactive groups which contributes to a different antioxidant power. The authors claimed that phenolic compounds had the strongest correlation (r> 0.95) with the four antioxidant properties, followed by flavonoids (r > 0.67) and saponins (r > 0.6). (Ngo *et al*., 2017)

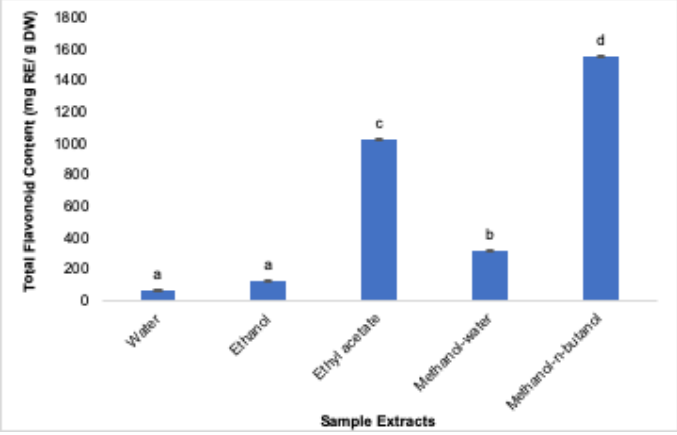

Fig. 3 Total flavonoid contents of five *M. charantia* extracts. Results were expressed as mean ± SD; (n = 3). Columns with different superscript letters (a, b, c, d) are significantly different from each other ($p < 0.05$). RE: Rutin equivalents.

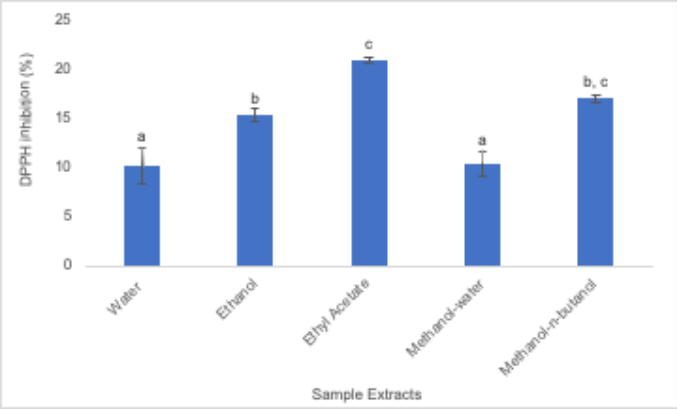

Fig. 4 DPPH radical scavenging activity of five *M. charantia* extracts (1 mg/ml). Results were expressed as mean ± SE; (n = 3). Columns with different superscript letters (a, b, c, d) are significantly different from each other ($p < 0.05$).

ABTS assay

Extract E was found to have the lowest IC_{50} of the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay whereas the weakest was by extract A. This findings were contradict with V. Le *et al*., (2018) which found that water extract exhibit the best ABTS capacity. However, ethanol extract showed a higher inhibition of the antioxidant activity in agreement with reports by Tan *et al*., (2014 c) which found highest value of ABTS from ethanol extract. There was no significant difference between the ABTS IC_{50} values for the ethyl-acetate and methanol-water extracts observed.

α-amylase Inhibitory Activity

The absorption of glucose was retarded through the inhibition of the duodenal carbohydrate-hydrolyzing enzymes such as α-amylase for anti-obesity and anti-diabetes therapeutic approach. The breakdown of α-1,4-glucosidic linkages of starch, glycogen and oligosaccharides into simpler monosaccharides for the intestinal absorption is catalyses by α-amylase. Dou *et al*., indicate that the inhibitory activities are influenced by both the saccharide moiety at C-3 and the glucose unit at C-28 of the aglycone which in this study might be the structure-activity-relationship involved with different extract. The α-Amylase inhibition percentage of all extracts at 40 ug/ml ranged from 3.73–39.30 % where highest inhibition was from Extract E (Methanol-n-butanol) and the lowest inhibition was from extract B (Ethanol). This findings is in agreement with Yue *et al*., (2017) which found around 20 % inhibitory activity by ethanol extract. However, the MC ethanol extract inhibitory activity of α-Amylase reported by Poovitha & Parani (2016) was found to be much higher (66.5 %). This may be due to the different extraction method done by the researcher which includes the usage of different polarity of solvent such as acid and acetone, and much more concentrated extracts (2.5 mg/ml).

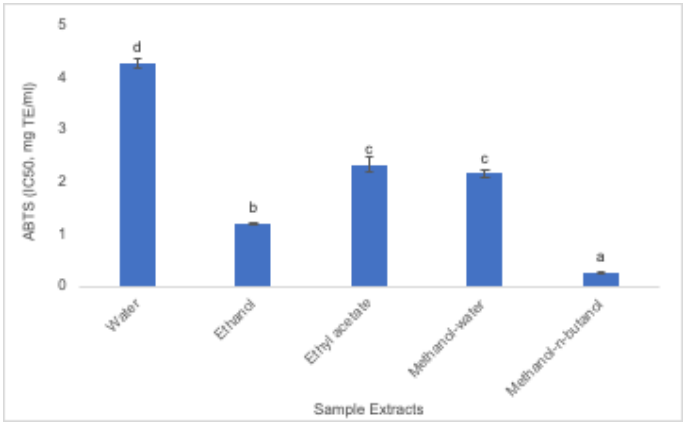

Fig. 5 ABTS activity of five *M. charantia* extracts. Results were expressed as mean ± SD; (n = 3). Columns with different superscript letters (a, b, c, d) are significantly different from each other ($p < 0.05$). TE: Trolox equivalents.

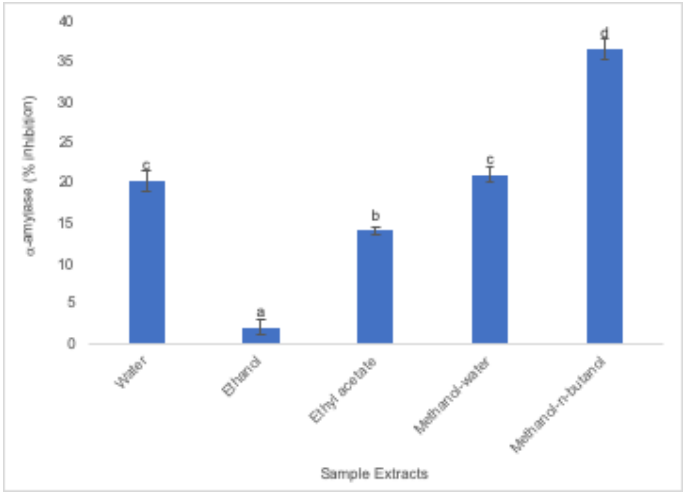

Fig. 6 α-Amylase inhibitory activities of five extracts of *M. charantia* tested at a concentration of 40 ug/mL. The values are expressed as mean ± SD (n = 3). Columns with different superscript letters (a, b, c, d) are significantly different from each other ($p < 0.05$).

Lipase Inhibitory Activity

The pancreatic lipase inhibition by ethyl acetate, methanol-water and methanol-n-butanol extracts were significantly stronger than ethanol and water extracts. Oishi *et al*., (2007) recorded that the combination of methanol and n-butanol fraction were strongly inhibit the activity of lipase compared to water. On the other hand, ethanol MC extracts were found to least inhibit pancreatic lipase activity as reported by Sahib *et al*., (2011).

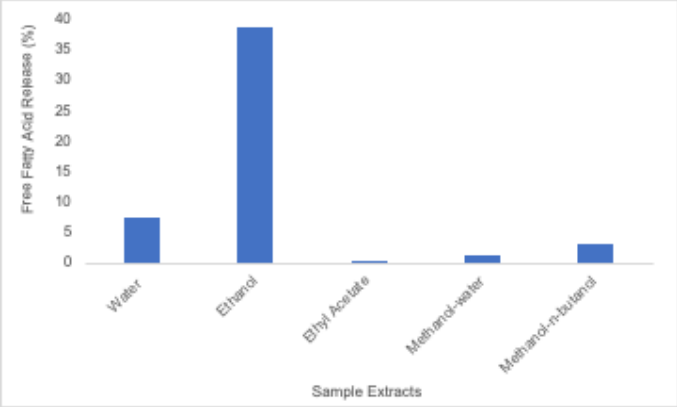

Fig. 7 Lipase inhibitory activities of five extracts of *M. charantia*.

CONCLUSION

In conclusion, the semi polar methanol-n-butanol is recommended as the solvent of choice, for extracting saponin and phenolic compounds effectively, inhibit the activities of α-amylase and exhibit significant antioxidant activities from *M. charantia* L. fruits. Taken together, the methanol-n-butanol solvent was found to be an effective solvent combination for the extraction of saponin from *M. charantia*. The *M. charantia* extracts showed inhibition of both α-amylase and lipase activities which may suggest the therapeutic potential of *M. charantia* for obesity and diabetes.

ACKNOWLEDGEMENT

This work was financially supported by Research University Grant from Universiti Teknologi Malaysia (Q.J130000.2546.14H62 and Q.J130000.2509.18H73).

REFERENCES

Akanni, O. O., Owumi, S. E., Adaramoye, O. A. 2014. In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from Artocarpus altilis, Ficus exasperate and Kigelia africana. *Asian Pacific journal of tropical biomedicine*, *4*, S492-S499.

Akter, S., Netzel, M. E., Tinggi, U., Osborne, S. A., Fletcher, M. T., Sultanbawa, Y. 2019. Antioxidant rich extracts of Terminalia ferdinandiana inhibit the growth of foodborne bacteria. *Foods*, *8*(8), 281.

Barve, K. H., Laddha, K. S., Jayakumar, B. 2010. Extraction of saponins from Safed Musli. *Pharmacognosy Journal*, *2*(13), 561-564.

https://umsc.my/?umsc_news=malaysia-has-become-fattest-country-in-asia-in-30-years. 15 May 2020.

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D., Vuong, Q. V. 2017. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. *Journal of Food Quality*, *2017*.

Oishi, Y., Sakamoto, T., Udagawa, H., Taniguchi, H., Kobayashi-Hattori, K., Ozawa, Y., Takita, T. 2007. Inhibition of increases in blood glucose and serum neutral fat by Momordica charantia saponin fraction. *Bioscience, biotechnology, and biochemistry*, *71*(3), 735-740.

Perez, J. L., Jayaprakasha, G. K., Patil, B. S. 2019. Metabolite profiling and in vitro biological activities of two commercial bitter melon (*Momordica charantia* Linn.) cultivars. *Food chemistry*, *288*, 178-186.

Poovitha, S., Parani, M. 2016. "In vitro and in vivo α-amylase and α-glucosidase inhibiting activities of the protein extracts from two varieties of bitter gourd (Momordica charantia L.)." *BMC complementary and alternative medicine* 16(1), 1-8.

Rodgers, R. J., Tschöp, M. H., Wilding, J. P. 2012. Anti-obesity drugs: past, present and future. *Disease models & mechanisms*, *5*(5), 621-626.

Sahib, N. G., Hamid, A. A., Kitts, D., Purnama, M., Saari, N., Abas, F. 2011. The effects of Morinda citrifolia, Momordica charantia and Centella asiatica extracts on lipoprotein lipase and 3t3-l1 preadipocytes. *Journal of Food Biochemistry*, *35*(4), 1186-1205.

Shobha, C. R., Prashant, V., Akila, P., Chandini, R., Suma, M. N., Basavanagowdappa, H. 2017. Fifty percent ethanolic extract of Momordica charantia inhibits adipogenesis and promotes adipolysis in 3T3-L1 pre-adipocyte cells. *Reports of biochemistry & molecular biology*, *6*(1), 22.

Srinivasulu, S., Pallavi, Y., Devi, B. G., Jyothi, H. K. P. 2017. Phytochemical and HPTLC Studies on Fruit Extracts of Momordica cymbalaria Fenzl, a Medicinally Important Plant. *Notulae Scientia Biologicae*, *9*(3), 350-360.

Tan, S. P., Kha, T. C., Parks, S. E., Roach, P. D. 2016. Bitter melon (Momordica charantia L.) bioactive composition and health benefits: A review. *Food Reviews International*, *32*(2), 181-202.

Tan, S. P., Vuong, Q. V., Stathopoulos, C. E., Parks, S. E., Roach, P. D. 2014 a. Optimized aqueous extraction of saponins from bitter melon for production of a saponin-enriched bitter melon powder. *Journal of food science*, *79*(7), E1372-E1381.

Tan, S. P., Parks, S. E., Stathopoulos, C. E., Roach, P. D. 2014 b. Extraction of flavonoids from bitter melon. *Food and Nutrition Sciences*, *2014*.

Tan, S. P., Stathopoulos, C., Parks, S., Roach, P. 2014 c. An optimised aqueous extract of phenolic compounds from bitter melon with high antioxidant capacity. *Antioxidants*, *3*(4), 814-829.

V Le, A., E Parks, S., H Nguyen, M., & D Roach, P. 2018 a. Improving the vanillin-sulphuric acid method for quantifying total saponins. *Technologies*, *6*(3), 84.

V Le, A., E Parks, S., H Nguyen, M., & D Roach, P. 2018 b. Effect of solvents and extraction methods on recovery of bioactive compounds from defatted Gac (Momordica cochinchinensis Spreng.) seeds. *Separations*, *5*(3), 39.

Yaakob, H. 2010. *Understanding the effects of lipid and surfactants on the intestinal absorption of flavonoids* (Doctoral dissertation, University of East Anglia).

Yue, J., Xu, J., Cao, J., Zhang, X., Zhao, Y. 2017. Cucurbitane triterpenoids from Momordica charantia L. and their inhibitory activity against α-glucosidase, α-amylase and protein tyrosine phosphatase 1B (PTP1B). *Journal of Functional Foods*, *37*, 624-631.

Yun, J. W. 2010. Possible anti-obesity therapeutics from nature–A review. *phytochemistry*, *71*(14-15), 1625-1641.