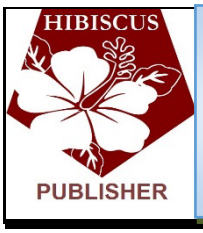
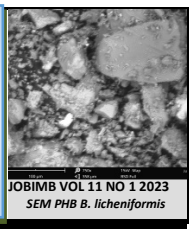
JOBIMB, 2023, Vol 11, No 1, 42-47
https://doi.org/10.54987/jobimb.v11i1.805



JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



Identification of Dipeptidyl-Peptidase 4 (DPP-4) Inhibitors from Miracle Berry Fruit (*Synsepalum dulcificum*) Extract

Nuraliana Najwa Hamsah Zollapi[1], Raihan Taib[1], Nurfatini Zakaria[1] and Mohd Ezuan Khayat[1]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

*Corresponding author:
Dr. Mohd Ezuan Khayat
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 Serdang,
Selangor,
Malaysia.
Email: m_ezuan@upm.edu.my

HISTORY

Received: 27th May 2023
Received in revised form: 21st June 2023
Accepted: 15th July 2023

KEYWORDS

DPP-4 Inhibitor
Miracle Berry Fruit
Type 2 Diabetes Mellitus
Silica Gel Chromatography
LC-MS/MS

ABSTRACT

Dipeptidyl-peptidase 4 is a novel targeted enzyme in type 2 diabetes mellitus (T2DM) therapy due to its regulatory effect on incretin; glucagon-like peptide 1 (GLP-1) that responsible for stimulating insulin and suppressing glucagon secretion. DPP-4 degrades GLP-1 to biologically inactive fragments. In incretin-based therapies, DPP-4 inhibitor helps to restrain the inactivation of GLP-1, which in turn prolonging GLP-1's half-life in blood and subsequently reducing the blood sugar more effectively. Nowadays, researchers are focusing to replace the synthetic drugs with natural-based drugs since they exert less toxicity. In our attempt to discover the natural inhibitor for DPP-4, miracle berry fruit was sequentially extracted with different solvents such as hexane, dichloromethane, chloroform, ethyl acetate and methanol and screened for the DPP-4 inhibitory activity. The analyses on the extractions show dichloromethane yielded the highest number of phenolic compounds with 38.37 mg GAE/g, while the highest number of flavonoids was found in hexane extract with 142.269 mg QE/g. On the other hand, qualitative analyses showed that methanol was able to extract alkaloids, terpenoids, saponins, tannins, quinones and carbohydrate from miracle berry fruit. It was also found that ethyl acetate extract was able to completely inhibit the enzyme. For further identification of constituent responsible for DPP-4 inhibitory activity, ethyl acetate extract was then subjected to silica gel column chromatography and eluted with different solvents with increasing polarity. The active fraction was then subjected to LC-MS/MS analysis and the results show it contains astragalin, nialamide, n-acetyl-l-phenylalanine, (+)-6-gingerol, 9-nitro oleate and 4(3H)-quinazolinone. The study indicates that miracle berry fruit extract contains natural DPP-4 inhibitor that can potentially be used for treating T2DM.

INTRODUCTION

Being the most common and well-known form of diabetes, type 2 diabetes mellitus (T2DM) is characterised by insulin resistance which leads to high blood sugar and associated with lack of insulin. Over the past decades, the commonness of type 2 diabetes has drastically increased which has caused high morbidity, mortality and healthcare costs [1]. In Malaysia, the commonness of T2DM is also worrisome as the amount of people diagnosed with the disease has been raising very quickly. The study done by National Health and Morbidity Surveys (NHMS) on the prevalence of T2DM among adults in the year 1996, 2006, 2011 and 2015 indicates that TD2M incidents have increased for the past two decades in Malaysia.

In recent years, incretin-based therapies have appeared as important agents in the treatment of T2DM. One of the classes of incretin-based therapies available is dipeptidyl peptidase-4 (DPP-4) inhibitors. DPP-4 inhibitors are new oral glucose-lowering agents, commonly named as incretin enhancers, which may be used as monotherapy or conjointly with additional anti-diabetic compounds [2]. They target the incretin system. After ingesting a meal, GLP-1 and GIP are being released from intestinal cells to blood circulation to promote insulin secretion in pancreatic β-cells [1]. These incretins however have short

half-life since they are degraded by DPP-4. Therefore, the presence of DPP-4 inhibitors can prolong their half-life thus stimulating more secretion of insulin.

Sitagliptin was the first approved selective inhibitor for DPP-4 followed by vildagliptin, saxagliptin, linagliptin and latest, alogliptin. However, DPP-4 inhibitors are also found to exert drawbacks. The study by Huang *et al*. reveals a total of 9706 adverse event reports that associate with DPP-4 inhibitors were identified from 2004 to 2019 [3]. Sitagliptin, saxagliptin, linagliptin, vildagliptin were associated with gastrointestinal nonspecific inflammation and dysfunctional conditions, hypersensitivity, severe cutaneous adverse reactions, and noninfectious diarrhea [3].

Looking at the adverse effects of currently used drugs in T2DM therapy, researcher shift their attention to natural compounds [4–6]. Nowadays, plant has been regarded as a rich source of medicine and growingly integrated into modern medicine. One of them is *Synsepalum dulcificum* that commonly known as miracle berry. When consumed, the fruit of miracle berry can change the sour prescription of foods into sweet. It contains greater level of vitamin C and phenolic antioxidants than other well-known berries such as blueberry and blackberry.

Rutin, epicatechin, quercetin, kaempferol, myricetin, gallic, ferulic, syringic acid, three tocopherols (α-tocotrienol, α- and γ-tocopherol), anthocyanins (delphinidin glucoside, cyanidin galactoside and malvidin galactoside) and lutein are among compounds that were discovered in the flesh of miracle berry. It was observed that the extract of the fruit of miracle berry exhibited greater inhibition towards lipid oxidation in the fish oil emulsion than gallic acid [7]. Due to the richness of its beneficial compounds, we hypothesised that the extract from miracle berry fruit could inhibit DPP-4.

Materials and Methods

Plant material

Synsepalum dulcificum fruits were collected from a plant nursery in Sepang, Selangor. The seed was separated from the fruit and freeze-dried before being pulverized into a fine powder.

Extraction method

Miracle berry powder (250 g) was sequentially macerated in 5 L hexane, dichloromethane, chloroform, ethyl acetate and methanol for 72 h each solvent. The extracts were then filtered using glass Whatman no.1 filter paper before evaporated in rotary evaporator. Prior to assays, all of extracts were dissolved to the concentration of 10 mg/mL in 1.5% DMSO except hexane and chloroform extracts which dissolved in 1.5% hexane and chloroform.

Determination of total phenolic content

Total phenolic content of the fruit extracts was quantified using Folin-Ciocalteu method according to Sembiring *et al*. with slight modifications [8]. Briefly, 25 µl of the extract was mixed with 125 µl of 0.2N Folin-Ciocalteu reagent and left to homogenize for 5 minutes. Subsequently, 100 µl of 7.5% sodium carbonate was added to the mixture. The absorbance was then measured at 760 nm against a blank using BioTek microplate reader (VT, USA) following the incubation of the mixture in the dark for 1 hour at room temperature. All assays were carried out in triplicate and dark environment. The total phenolics content of the extract was calculated by using a calibration curve obtained from gallic acid standard and expressed as mg gallic acid equivalent (GAE)/100 g FW.

$$TPC = \frac{\text{concentration of gallic acid from calibration curve } \left(\frac{\text{mg}}{\text{mL}}\right) \text{X volume of extract solution(mL)}}{\text{weight of extract (g)}}$$

Determination of total flavonoid content

Total flavonoid content was determined based on the method described by [9]. A hundred microliters of diluted extracts solution (1 mg/mL) were mixed with 10 µL of 5% sodium nitrite. The reaction mixture was incubated for 5 min in dark. 10 µL of 1 M sodium hydroxide was added to resulting mixture followed by the addition of 30 µL of distilled water. The absorbance was measured at 510 nm using a microplate reader. The total flavonoid content was calculated by using a calibration curve obtained from quercetin standard and expressed as mg quercetin equivalent (QE)/100 g FW. The TFC was determined using the following formula:

$$TFC = \frac{\text{concentration of quercetin from calibration curve } \left(\frac{\text{mg}}{\text{mL}}\right) \text{X volume of extract solution(mL)}}{\text{weight of extract (g)}}$$

Test for alkaloid

Two millilitres of extracts were added to 1 mL of Wagner's reagent that prepared by dissolving 2 g of iodine and 6 g of potassium iodide in 100 mL of distilled water. Formation of brown or reddish precipitate indicates the presence of alkaloids.

Test for carbohydrate

A few drops of Benedict's reagent were added to 2 mL of extracts and boiled in water bath. The formation of brick red precipitate indicates the presence of carbohydrates.

Test for saponin

Four millilitres of distilled water were added to 2 mL of extracts. Mixture was mixed and shaken vigorously. Formation of foam layer obtained on the top of the test tube indicates presence of saponins.

Test for sterol or triterpenoid

Two millilitres of extracts were treated with a few drops of concentrated sulphuric acid. Appearance of golden yellow or reddish-brown colour indicates presence of triterpenes.

Test for quinone

A few drops of 5% of potassium hydroxide were added to 3 mL of extracts. Formation of red colour in alkaline phase indicates presence of quinines.

Test for tannin

A millilitre of 5% (w/v) aqueous ferric chloride solution was added to 1 mL of extracts. The appearance of green, purple, blue or black colour indicates the presence of tannins.

DPP-4 Inhibitory assay

The DPP-4 inhibition activity of various solvent extracts was measured with the Cayman DPP-4 Inhibitor Screening Kit (MI, USA) according to the instructions of the manufacturer. In brief, a thirty microlitres of assay buffer, 10 µL of diluted DPP-4 enzyme and 10 µL of extract were mixed in 96-well plate. The reaction was initiated by the addition of 50 µL of diluted substrate to all the wells used. The plate was covered and incubated at 37°C for 30 min before read using BioTEK fluorescence multiplate reader (VT, USA) at excitation wavelength of 360 nm and emission wavelength of 460 nm. The percentage inhibition was calculated as follow:

$$\%\ \text{of inhibition} = \left[\frac{\text{Slope of initial activity} - \text{Slope of inhibitor)}}{\text{Slope of initial activity}}\right] x100$$

A commercial DPP-4 inhibitor, Sitagliptin at concentration of 100 µM was used as positive control.

Silica gel column chromatography fractionation

The miracle berry extract was subjected to silica gel column chromatography (24 cm × 5 cm) through a column of silica gel (0.040-0.063 mm mesh) and eluted with different ratios of hexane:ethyl acetate (C_6H_{12}:$CHCl_3$; 1:10) and methanol:ethyl acetate (CH_3OH:$CHCl_3$; 1:10). Table 3.1 shows different ratio of both mixtures used during fractionation process. The fractions with similar TLC profile will be pooled and evaporated using rotary evaporator.

Table 1. Solvent system ratios used for silica gel column Thin Layer Chromatography (TLC).

No	Solvents	Ratio
1	Hexane: Ethyl acetate (1:10)	100:0
2		95:5
3		90:10
4		85:15
5		80:20
6		75:25
7		70:30
8		65:35
9		60:40
10		55:45
11		50:50
12		45:55
13		43:57
14		40:60
15		38:62
16		36:64
17		34:66
18		32:68
19		30:70
20		28:72
21		26:74
22		24:76
23		22:78
24		20:80
25		18:82
26		16:84
27		14:86
28		12:88
29		10:90
30		8:92
32		6:94
33		4:96
34		2:98
35	Methanol: Ethyl acetate (1:10)	100:0

Briefly, a straight line was drawn gently approximately 0.8 cm from the bottom of silica plate. The spots of sample were then applied to the line Using TLC spotters made from glass capillary tubes. The spotted TLC plates were placed into the chambers containing hexane:ethyl acetate (1:10) by leaning them against the side of each of the chamber. The solvent was allowed to draw up the plate until it was approximately 0.5 cm from the end. The plates were removed and by using short-wave UV light, spots were examined.

LC-MS/MS analysis

The constituents of active fractions were identified using an AB Sciex 3200 QTrap LCMS/MS with a Perkin Elmer FX 15 UHPLC system (MA, USA). The postive ion mass spectra were obtained with a LC QTrap MS/MS detector in full ion scan mode (100 to 1200 for full scan and 50–1200 for MS/MS scan) at a scan rate of 0.5 Hz. The system was supported with mass spectrometry software and a spectral library provided by ACD labs (Toronto, ON, Canada). Analyte was separated using C18 column (4 × 250 mm, 5 μm, Phenomenex). The mobile phase consisted of water (solvent A) and methanol with 1% acetonitrile (solvent B), each containing 0.1% formic acid and 5 mM ammonium format. The gradient was set from 40% solvent B to 50% solvent B over 11.00 min at a flow rate of 1.0 mL/min. The corresponding peaks from the QTrap LCMS/MS analysis were identified by comparison with the literature/ACD labs mass spectral library.

Statistical analysis

Statistical analysis was done using GraphPad prism 9 (MA, USA). One-way analysis of variance with post hoc analysis by Tukey's test was used to compare between groups. P-value of < 0.05 is considered significant.

RESULTS AND DISCUSSIONS

Total phenolic content of miracle berry extracts

The total phenolic contents (TPC) of the miracle berry fruit extracted with hexane, dichloromethane, chloroform, ethyl acetate and methanol are shown in **Fig.** 1. The results show the best solvent for the extraction of phenolic compounds in miracle berry fruit was dichloromethane followed by methanol, chloroform, ethyl acetate, and hexane with the phenolic content values of 0.383±0.062 mg GAE/g, 0.221±0.001 mg GAE/g, 0.174±0.031 mg GAE/g, 0.171±0.002 mg GAE/g, and 0.072±0.002 mg GAE/g, respectively.

It has been reported that phenolic compounds were extracted more when polar organic solvents were used [10]. From the results obtained, it can be demonstrated that TPC of dichloromethane extract is higher than the methanol extract, even though methanol having higher polarity than dichloromethane. However, in line with the study by Bhebhe *et al*., it can be concluded that the extraction of phenolic compounds differs from plant to plant such that only a solvent well suited for a particular plant may extract more phenolics [11]. It is possible that a solvent perhaps is efficient on one plant and less efficient on another.

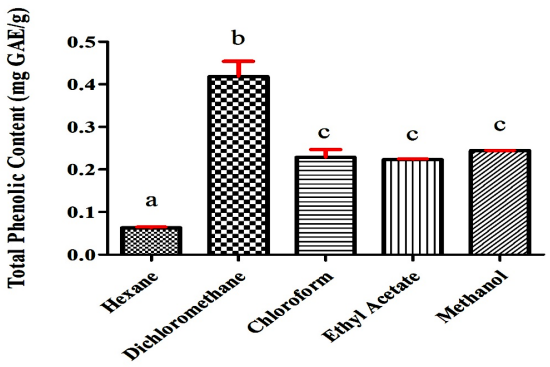


Fig. 1. Total phenolic content in various extracts of miracle berry (*S. Dulcificum)* fruit at concentration of 1mg/mL. Data are mean ± standard deviation and all determinations were performed in triplicates (n=3). Bars bearing of same letter are not significant by one-way ANOVA and Tukey's multiple comparison test, P <0.05. Results are expressed as gallic acid equivalents (GAE).

Total flavonoid content of miracle berry extracts

Total flavonoid content (TFC) of various solvent extracts were quantified using the aluminium chloride colorimetric method at 50 nm using quercetin as a standard. The results are shown in **Fig. 2**. Among the various solvent extracts, the highest amount of flavonoid content was found in hexane with value of 0.142.269±0.018 mg QE/g. No significant different was found between the TFC values of dichloromethane, chloroform, ethyl acetate, and methanol extracts (P<0.05). The usage of methanol

as an extraction solvent usually resulted in maximum flavonoid extraction since they are generally well extracted in polar solvent [12]. In contrast we found that non polar hexane was the best extraction solvent for flavonoid from miracle berry fruit which suggests that the fruit may majorly contain non-polar or less polar flavonoid such as isoflavones, flavanones, methylated flavones and flavanols [13]. Nonpolar flavonoids have affinity for solvents with lower polarity [14].

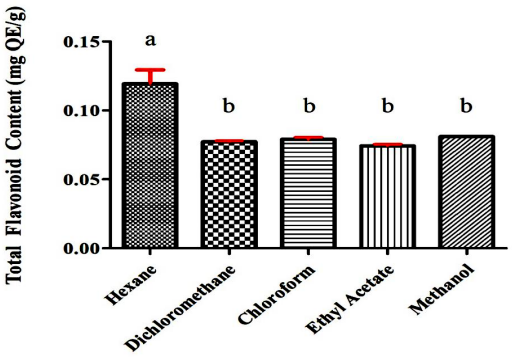


Fig. 2. Total flavonoid content in various extracts of miracle berry (*S. Dulcificum)* fruit at concentration of 1mg/mL. Data are mean ± standard deviation and all determinations were performed in triplicates (n=3). Bars bearing of same letter are not significant by one-way ANOVA and Tukey's multiple comparison test, P <0.05. Results are expressed as quercetin equivalents (QE).

Phytochemical screening of miracle berry fruit extracts

Unlike commonly known berries which their phytochemicals were extensively studied, the phytochemical profile of miracle berry is still not well documented. Miracle berry was proven to be a great source of antioxidant-rich phytochemicals comparable with blueberry, blackberry and most known berries [7]. In this study, test for alkaloids, terpenoids, saponins, tannins, quinines and carbohydrates had been done on miracle berry fruit of various solvent extracts.

Table 1 shows the qualitative analysis of phytochemicals content in miracle berry fruit extracts**.** According to the results, methanol was able to extract all the phytochemicals that were tested, which are alkaloid, terpenoid, saponin, tannin, quinine and carbohydrate. It was observed that the presence of phytochemicals in the extracts was decreased when the polarity of solvents was reduced. This observation might be due to fact that the majority components of plant are polar [15]. Therefore, it explains the effectiveness of methanol to extract wide range of phytochemicals in miracle berry fruit as it was most polar solvent that being tested in this study.

Ethyl acetate was the second most polar solvent in this study and it has successfully extracted alkaloid, tannin, quinone and carbohydrate but not terpenoid and saponin. As for dichloromethane and chloroform, there are only two phytochemicals that were positively detected in their extracts. Dichloromethane extract showed positive result in alkaloid and saponin tests whereas chloroform extract showed positive result in alkaloid and terpenoid tests.

The observation suggests that the polarity of the solvent determines type, composition and anti-oxidant activity of phytochemical of the extracts [16]. Finally, hexane extract showed negative results in the test for all phytochemicals. The results further emphasize the ineffectuality of less polar solvent to extract phytochemicals from plant. Together, despite some phytochemicals being short of in some solvent extracts, the findings confirmed that miracle berry fruit as a whole contains all the phytochemicals screened for which is in agreement with Du and team on miracle berry fruit being a great source of antioxidant-rich phytochemicals [7].

Table 2. Qualitative analysis of phytochemicals content in miracle berry fruit extract in various solvents.

Phytochemicals	Extracts				
	Hexane	Dichloro methane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	+	+	+	+
Terpenoids	-	-	+	-	+
Saponins	-	+	-	-	+
Tannins	-	-	-	+	+
Quinones	-	-	-	+	+
Carbohydrates	-	-	-	+	+

Note: - = negative result, + = positive result.

Screening of DPP-4 inhibitory activity in miracle berry fruit extracts

The results demonstrate that among different solvent extracts studied, ethyl acetate extract shows the highest DPP-4 inhibitory activity with 100% of enzyme inhibition (**Fig.** 3). The efficiency of solvents extracts in inhibiting DPP-4 is in this order: ethyl acetate extract> methanol extract> dichloromethane extract> chloroform extract> hexane extract. The efficacy of the ethyl acetate extract to inhibit DPP-4 enzyme activity similarly to 100 µM sitagliptin indicates that constituent of the berry can act as a naturally occurring DPP-4 inhibitor. This high enzyme inhibition of the extract could also be due to the capability of ethyl acetate as a polar solvent to extract polar phytochemicals which believed to abundant in plant.

Methanol, on the other hand, gave the second highest percentage of inhibition with value of 29% despite being the most polar solvent. It may cause by the fact of compounds that inhibit DDP-4 are not readily extracted by methanol. Compounds that are being extracted differ from plant to plant such that only a solvent well suited for a particular plant may extract more of the particular compounds [11]. Lastly, dichloromethane, chloroform and hexane extract just slightly inhibited DPP-4 with 22 %, 8 % and 7.76 % of enzyme inhibition, respectively.

Du *et al*. [7] reported that miracle berry has much higher total phenolic content than other reported berry fruits such as blackberry, blueberry, Corema album berry or strawberry. The compounds in the berry family were discovered to have the ability to inhibit DPP-4 with remarkable activity in terms of IC_{50} . Anthocyanins isolated from blueberry-blackberry wine blends were also found to have inhibitory effects on DPP-4 [17]. In another study Fan et al. observed that out of 27 phenolic compounds commonly present in berries, 16 found to inhibit DPP-4 [18]. Some of these compounds also present in miracle berry fruit.

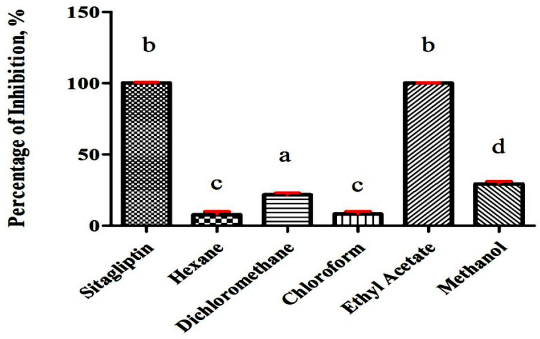


Fig. 3. The inhibitory activity for DPP-4 by various solvent extracts of miracle berry (*S. Dulcificum)* fruit at concentration of 1 mg/mL. Data are mean ± standard deviation and all determinations were performed in triplicates (n=3). Bars bearing of same letter are not significant by one-way ANOVA and Tukey's multiple comparison test, P <0.05. Results are expressed as percentage (%).

Identification of DPP-4 inhibitors in miracle berry fruit extract

In order identify the responsible compounds for DPP-4 inhibitory activity in miracle berry fruit, the extract was subjected to silica gel column chromatography. There were 309 fractions was collected at the end of chromatography. There were then pooled based on TLC profile that resulting 8 pooled fractions named F1, F2, F3, F4, F5, F5, F6, F7 and F8. These pooled factions were then evaporated using rotary evaporator, however, for F7, the solvent was failed to be completely removed, thus it was collected and labeled as mother liquor (ML).

The results obtained demonstrated that ML showed the highest DPP-4 inhibition which inhibiting 48% of DPP-4 activity (**Fig. 4**). It should be noted that in screening study ethyl acetate extract completely inhibited DPP-4 activity, however, after chromatography, the inhibition was reduced to half. This might be due to the degradation of bioactive compound during the isolation process. Next, LC-MS/MS was used to identify constituents of ML. As shown in Table 3 and Table 4, a total of six constituents identified which were detected by the LC-MS/MS in both positive and negative mode. The compounds like astragalin, nialamide, n-acetyl-l-phenylalanine and (+)-6-gingerol were previously reported to possess DPP-4 inhibitory activity [19–21].

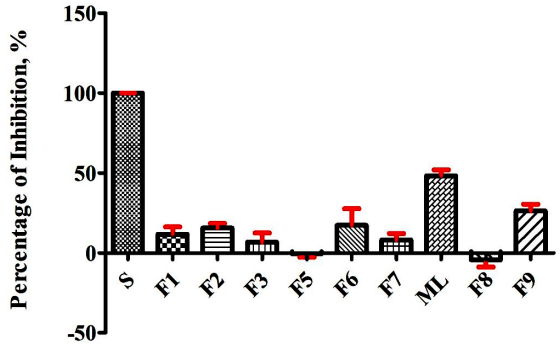
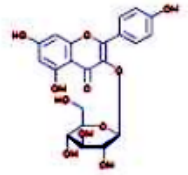
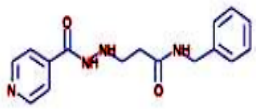
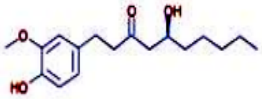


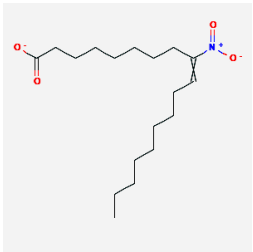
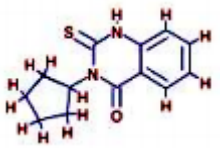
Fig. 4. The inhibitory activity for DPP-4 by various solvent extracts of miracle berry (*S. Dulcificum)* fruit at concentration of 1mg/mL. Data are mean ± standard deviation and all determinations were performed in triplicates (n=3). Results are expressed as percentage (%).

Table 3. Data obtained from LC-MS/MS on compounds in mother liquor with inhibitory activity towards DPP-4 in the negative mode.

Compound Name	RT (min)	MW	Structure
Astragalin	6.33	488.1001	
Nialamide	3.52	298.1417	
N-Acetyl-L-phenylalanine	4.80	207.0890	
(+)-[6]-Gingerol	13.27	294.1830	

Note: RT= Retention time, MW= Molecular weight

Table 3. Data obtained from LC-MS/MS on compounds in mother liquor with inhibitory activity towards DPP-4 in the positive mode.

Compound Name	Rt (Min)	Mw	Structure
9-Nitro Oleate	2.25	181.1253	
4(3h)-Quinazolinone	6.08	246.0832	

Note: RT= Retention time, MW= Molecular weight

CONCLUSION

The study shows ethyl acetate was the best solvent to extract naturally occurring DPP-4 inhibitor from miracle berry fruit. The efficacy of the extract to inhibit DPP-4 enzyme activity was comparable to sitagliptin suggesting it potentially can be used as natural alternative for DPP-4 inhibitor. The identification of compounds in the ethyl acetate extracts confirms the presence of several compounds that previously reported to inhibit DPP-4 such as astragalin, nialamide, n-acetyl-l-phenylalanine, and (+)-

6-gingerol. The information from this study potentially can be useful in diabetes management.

ACKNOWLEDGEMENT

This project was funded by Geran Putra Inisiatif Putra Siswazah (UPM/700-2/1/GP-IPS/9601700) dan Geran Putra Inisiatif Putra Muda (UPM/FBSB/700-2/1/GP-IPM/955600) from Universiti Putra Malaysia.

REFERENCES

1. Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology. 2007;132(6):2131–57.
2. Scheen AJ. Pharmacokinetics of dipeptidylpeptidase-4 inhibitors. Diabetes Obes Metab. 2010;12(8):648–58.
3. Huang J, Jia Y, Sun S, Meng L. Adverse event profiles of dipeptidyl peptidase-4 inhibitors: Data mining of the public version of the FDA adverse event reporting system. BMC Pharmacol Toxicol. 2020;21(1).
4. Zarib ANM, Zollappi ANH, Abidin MZ, Rahim MBHA, Gani SA, Hamid M, et al. Total phenolic, total flavonoid and antioxidant activity of extract from rhizome of cibotium barometz prepared by various solvents. Malays J Biochem Mol Biol. 2018;21(2):17–22.
5. Abidin MZ, Yatim NLAM, Zollapi NNH, Rahim MBHA, Gani SA, Hamid M, et al. Optimization of ultrasonic-assisted extraction of phenolic compound from golden chicken fern (Cibotium barometz) rhizome via response surface methodology. Malays J Biochem Mol Biol. 2019;22(1):87–92.
6. Zainudin RF, Abidin MZ, Hamsah Zollappi AN, Mohd Yatim NLA, Tamsir NM, Hamid M, et al. Enhancement of anti-advance glycation end product formation and antioxidant activity of salak peel extracts using betaine-based deep eutectic solvents. Malays J Biochem Mol Biol. 2020;23(2):106–12.
7. Du L, Shen Y, Zhang X, Prinyawiwatkul W, Xu Z. Antioxidant-rich phytochemicals in miracle berry (Synsepalum dulcificum) and antioxidant activity of its extracts. Food Chem. 2014 Jun;153:279–84.
8. Sembiring EN, Elya B, Sauriasari R. Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of Caesalpinia bonduc (L.) Roxb. Pharmacogn J. 2018;
9. Chang C chi, Yang M hua, Wen H mei, Chern J chuan. Estimation of Total Flavonoid Content in Propolis by. J Food Drug Anal. 2002;10(3):7802.
10. Kaczorová D, Karalija E, Dahija S, Bešta-Gajević R, Parić A, Ćavar Zeljković S. Influence of extraction solvent on the phenolic profile and bioactivity of two achillea species. Molecules. 2021;26(6):1–15.
11. Bhebhe M, Füller TN, Chipurura B, Muchuweti M. Effect of Solvent Type on Total Phenolic Content and Free Radical Scavenging Activity of Black Tea and Herbal Infusions. Food Anal Methods. 2016 Apr;9(4):1060–7.
12. Turkmen N, Velioglu Y, Sari F, Polat G. Effect of Extraction Conditions on Measured Total Polyphenol Contents and Antioxidant and Antibacterial Activities of Black Tea. Molecules. 2007 Mar;12(3):484–96.
13. Andersen ØM, Markham KR. Chemistry, Biochemistry and Applications Edited by. 2006.
14. Rodríguez De Luna SL, Ramírez-Garza RE, Serna Saldívar SO. Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. Sci World J. 2020;2020.
15. Sri Widyawati P, Dwi Wibawa Budianta T, Anggraeni Kusuma F, Livia Wijaya E. Difference of Solvent Polarity To Phytochemical Content and Antioxidant Activity of Pluchea indicia Less Leaves Extracts. Available Online Wwwijpprcom Int J Pharmacogn Phytochem Res. 2014;6(4).
16. Dehkharghanian M, Adenier H, Vijayalakshmi MA. Study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. Food Chem. 2010 Aug;121(3):863–70.
17. Gao Y, Zhang Y, Zhu J, Li B, Li Z, Zhu W, et al. Recent progress in natural products as DPP-4 inhibitors. Future Med Chem. 2015 Jun;7(8):1079–89.
18. Fan J, Johnson MH, Lila MA, Yousef G, de Mejia EG. Berry and Citrus Phenolic Compounds Inhibit Dipeptidyl Peptidase IV: Implications in Diabetes Management. Evid-Based Complement Altern Med ECAM. 2013 Aug;2013:479505.
19. Tanwar O, Deora GS, Tanwar L, Janardhan S, Alam MM, Shaquiquzzaman M, et al. Novel hydrazine derivatives as selective DPP-IV inhibitors: Findings from virtual screening and validation through molecular dynamics simulations. J Mol Model. 2014;20(4).
20. Samad M Bin, Mohsin MNA Bin, Razu BA, Hossain MT, Mahzabeen S, Unnoor N, et al. [6]-Gingerol, from Zingiber officinale, potentiates GLP-1 mediated glucose-stimulated insulin secretion pathway in pancreatic β-cells and increases RAB8/RAB10-regulated membrane presentation of GLUT4 transporters in skeletal muscle to improve hyperglycemi. BMC Complement Altern Med. 2017;17(1):1–13.
21. Cid-Ortega S, Monroy-Rivera JA. Extraction of kaempferol and its glycosides using supercritical fluids from plant sources: A review. Food Technol Biotechnol. 2018;56(4):480–93.