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Mimosa pudica L.: A comparative study via *in vitro* analysis and GC Q-TOF MS profiling on conventional and supercritical fluid extraction using food grade ethanol

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The present study compared conventional (maceration) extraction ($EtOH_{conv}$) and supercritical fluid extraction (SFE) methods as a mean of comparing conventional and green process for a weed namely *Mimosa pudica* L. to obtain a safe antidiabetic natural agent. *In vitro* analysis comprised of two different assays, antioxidant assay (determination of total phenolic content, total flavonoid content, and 2,2-diphenyl-1-picrylhydrazyl assay) and antidiabetic assay (inhibition of α-amylase and α-glucosidase). GC Q-TOF MS profiling for both extracts was done after derivetisation to confirm the presence of bioactive compounds. SFE was performed at 40 MPa pressure, 60 °C temperature and 5 mL/min CO_2 flow rate using 30 % ethanol (co-solvent) for 2 h. $EtOH_{conv}$ prepared using 95 % ethanol through conventional method (maceration) showed a good *in vitro* antioxidant potential and digestive enzymes inhibitory effect compared to supercritical fluid extract. α-amylase and α-glucosidase inhibitory activities for $EtOH_{conv}$ at 1 mg/mL were 30.08 % (±5.23) and 38.29 % (±2.52), whereas for standard acarbose it was 28.24 % (±13.66) and 36.93 % (±2.70), respectively. Supercritical fluid extract showed less potent *in vitro* antioxidant and digestive enzymes inhibitory effects (15.67±4.03- α-amylase, 28.36±2.01- α-glucosidase). GC Q-TOF MS analysis was done to confirm the presence of bioactive compounds in both the extracts. Although $EtOH_{conv}$ showed better results, SFE was found to contain more bioactive compounds associated with various pharmacological effects especially antioxidative as per GC Q-TOF MS results. SFE being a clean and green technology could be employed in future with more focus on method development and optimization to reproduce better and safe bioactive products from the neglected weed *M. pudica*.

Keywords: Antioxidant, Conventional extraction, Derivetisation, GC Q-TOF MS, *Mimosa pudica* L., SFE.

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Introduction

Global consumer demands for food, especially of natural organic quality containing natural additives have escalated exponentially in recent years with increasing health awareness among people worldwide[1]. Antioxidants have been especially demanded to be either naturally present in high amount or purposefully added to the food to enhance the quality of food that act as free radical fighting agents, thereby increasing the shelf life[2]. This demand has prompted many researchers to find new antioxidant rich sources, from which the potent antioxidants could be extracted out effectively[3]. In conjunction with the problem to tackle this key issue, alternative sources have been sought to overcome the escalated demand for the production of high quality of food that could prove beneficial for mankind. Alternative sources include biomass of lignocellulosic material namely agricultural and forest wastes, weeds, and seaweeds, which are renewable, cheap, and abundant in nature[4].

In order to extract medicinally active ingredients from such waste, the demand for clean extraction is a great choice to reduce toxin level and organic traces[5]. In lieu of conventional methods, various green technologies are being developed and employed throughout the world to address this issue constructively with regard to recover toxin free medicinally active agents or extracts. Green technologies include supercritical fluid extraction (SFE), sub-critical extraction (SCE), pressurised liquid extraction (PLE) among others[6]. In the current study, SFE has been employed in extraction process that utilises eco-friendly solvent system like CO_2 along with co-solvent particularly food grade bio-solvent namely ethanol. Despite using an organic

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solvent, the SFE process is still deemed green, since the amount of an organic solvent used is very little and the solvent is considered as food grade. This method allows scientists to work with various parameters like temperature, pressure, flow rate of co-solvent, and carbon dioxide to get toxin free extract of medicinal significance[7].

***Mimosa pudica* L.**

M. pudica var. *hispida* Bren. belongs to the family Fabaceae-Mimosoideae or Mimosaceae and is commonly known as shameful plant, sensitive plant, touch me not, bashful weed. Found mostly in Southeast Asia, it is a very common, self-growing, pan-tropical weed found in farming land, wasteland, grassy area, forest floor, hill, and marshland. However, it is grown throughout the tropics now and is now termed as pan-tropical weed. It is a low growing invasive shrub with prickly stem and thorns adorning the aerial parts; flowers are usually pink or purple in colour[8].

This weed is traditionally used in Bangladesh, India, Philippine, China, and Malaysia to treat disorders like diabetes mellitus, cardiovascular diseases (CVS); is used as a wound healing, anxiolytic, nerve regenerator, and antimicrobial agent; and also in birthcontrol[9]. Chemical profiling and phytochemical investigations have found this weed to be rich in phenolics, glycosides, flavonoids, and alkaloids[10]. In this study, food grade ethanol was chosen as the extracting solvent and comparison has been made between two extraction techniques for their extractability to yield potential antioxidant and antidiabetic components from this weed for their probable use in the management of diabetes. Chemical profiling using gas chromatography mass spectra coupled t o quadruple time of flight detection system known as GC Q-TOF MS, *in vitro* antioxidant potency (using total phenolic content, total flavonoid content, and DPPH assays), and diabetic (digestive) enzyme inhibition assay using α-amylase and α-glucosidase have been carried out to explore its potential in the efficacious management of diabetes in future.

Materials and Methods

Chemicals

DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin Ciocalteu reagent and solvents were bought from Fisher and Merck. The enzymes (α-amylase type iv- B from porcine pancreas and α-glucosidase type 1 from baker's yeast), p-nitrophenyl-α-D-glucopyranoside (p-NDG), 3,5-dinitro salicylic acid (DNS), potato starch, sodium phosphate, potassium phosphate monobasic, dipotassium phosphate, NaCl, Na_2CO_3, were bought from Sigma-Aldrich. Ultra-pure water was used wherever water was needed as the solvent. UV-Vis Microplate Spectrophotometer from Tecan Nano Quant, Infinite M200, Austria was used. Commercial grade liquid carbon dioxide (99.99 % pure) supplied in cylinder was purchased from Malaysian oxygen (MOX), Malaysia.

Plant material

Fresh aerial parts of *M. pudica* were collected during flowering season (March-July 2013) from the vicinity of the International Islamic University Malaysia campus (IIUM), Kuantan, Malaysia. The plant was identified by Taxonomist of KOP, IIUM, Malaysia and a voucher specimen (NMPC-QU037) has been deposited in the Herbarium, Faculty of Pharmacy, IIUM, Malaysia for future references.

Conventional extraction

The fresh aerial parts of *M. pudica* were dried in a PROTECH laboratory air dryer (FDD-720-Malaysia) at 40 °C for 7 days and pulverized using Fritsch Universal Cutting Mill-PULVERISETTE 19-Germany. It was then stored in a desiccator until further use. The coarsely ground dry powder (500 g) was macerated with food grade 95 % ethanol in a round bottom flask and subjected to 3 cycles (each 24 h soaking) of ethanol extraction (2.5 L) at room temperature followed by 4 cycles of heat under reflux (2 h each) over boiling water bath to ascertain maximum yield of bioactive compounds. Later, the ethanol extract ($EtOH_{conv}$) obtained through conventional maceration process was filtered and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210, Switzerland)[3].

Supercritical fluid extraction

Supercritical fluid extraction (SFE) system was comprised of CO_2 cylinder, cool water circulator (VTR-620, Jeio Tech., Seoul, Korea), column thermostat (CO-1560, JASCO Corporation, Tokyo, Japan), solvent (CO_2 and modifier) pumps (PU-1580, JASCO), UV/VIS detector (UV-1575, JASCO), back pressure regulator (880-81, JASCO) and water bath (HeidolphWB-2000). About 10 g powdered sample of *M. pudica* was kept in the extraction vessel and placed in the column thermostat set at 60 °C. Pressure (40 MPa) was adjusted at the back pressure regulator

and solvent pumps. The flow rates for CO_2 and % modifier were fixed at 5 mL/min and 30 %, respectively. Once the set temperature and pressure (at solvent pumps and backpressure regulator) were achieved after turning on the injection valve and the system was in equilibrium, the extraction was carried out for 2 h. Extract was collected in an amber vial placed at the collector and later stored at 4 °C before further analysis for the extract yield and bioactive components. The percentage extract yield was measured by drying the liquid extract at 70 °C until constant weight of the extract was obtained[11].

***In- vitro* analysis**

DPPH free radical scavenging activity

DPPH assay was performed to determine the radical scavenging potential of a sample with respect to finding out its inhibitory effect on the free radical in the form of DPPH. The conventional ethanol extract ($EtOH_{conv}$) and SFE extract were evaluated for their free radical scavenging activity by following the method described by Nickavar *et al.* with some modifications[12]. Absorbance was measured using UV-Vis spectrometer at 517 nm. Quercetin was used as a standard and proper blanks were used. IC_{50} values in µg/mL were determined for the samples and the standard deviation was calculated. DPPH as percentage scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging activity} = \frac{(\text{Control - Absorbance})}{\text{Control}} \times 100$$

Total phenolic content (TPC) assay

This assay was performed to determine the total amount of phenolic compounds present in the sample with respect to a standard phenolic compound namely gallic acid. TPC was determined using Folin Ciocalteu method by following the procedure described by Singleton and Rossi[13]. Absorbance was measured using UV-Vis spectrometer at 765 nm. Experiments were performed in triplicate and gallic acid was used as a standard. TPC was calculated using the following equation:

$$\text{TPC (mg/g)} = \text{GAE} \times \text{V} \times \frac{(\text{Dx}10^{-6} \times 100)}{\text{Sw}}$$

where GAE is gallic acid equivalent (mg), V is volume of sample (mL), D is dilution factor, and Sw is sample weight (g).

Total flavonoid content (TFC) assay

This assay was followed to determine the total amount of flavonoids present in a particular sample with respect to a standard flavonoid (Quercetin). TFC assay was performed using $AlCl_3$ colorimetric method (as described by Zhishen *et al.*)[14]. Absorbance was measured using UV-Vis spectrometer at 415nm. Quercetin was used as a standard and appropriate blanks were used. Experiments were done in triplicate. TFC was calculated using the following equation:

$$\text{TFC (mg/g)} = \text{QExVx} \frac{(\text{Dx}10^{-6}\text{x}100)}{\text{Sw}}$$

where QE is quercetin equivalent (mg), V is volume of sample, D is dilution factor, and Sw is sample weight (g).

α-amylase inhibitory assay

Standard protocols from Worthington were followed with some modifications to carry out α-amylase inhibitory assay[15]. Briefly, in 96-well plate, 25 µL of $EtOH_{conv}$ and SFE extracts at (1 mg/mL) were added to 25 µL of (0.5 mg/mL) α-amylase enzyme in 20 mM sodium phosphate buffer (pH 6.9, with 6.7 mM NaCl). The mixture was incubated for 10 min at 25 °C followed by addition of 25 µL of 0.5 % starch in phosphate buffer. The mixture was further incubated at 25 °C for 10 min. Subsequently, reaction was stopped using 50 µL of DNS solution and incubated over water bath at 100 °C for 5 min. Readings were taken using microplate reader at 540 nm. Blank was the extract with buffer instead of enzyme and control was solvent in place of extract and enzyme. Acarbose at 1 mg/mL (in sodium phosphate buffer) was used as standard. Enzyme inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{AbsS}}{\text{AbsE}}\right) \text{x } 100$$

where AbsS is the absorbance of sample and AbsE is the absorbance of enzyme.

α-glucosidase inhibitory assay

Standard protocol from Apostolidis *et al.* was followed with some modifications to carry out α-glucosidase enzyme inhibitory assay[16]. Briefly, in 96-well plate, 50 µL of $EtOH_{conv}$ and SFE extracts at 1 mg/mL was added to 100 µL of (l U/mL) α-glucosidase enzymes (Sigma-Aldrich) in 0.1 M

potassium phosphate buffer (pH 6.9). The mixture was incubated at 25 °C for 10 min after which 50 µL of ρNDG was added at 5 s intervals and further incubated at 25 °C for 5 min. Readings were taken using microplate reader at 405 nm. Blanks were prepared with the extracts and solvents instead of enzyme and control was consisting of enzyme and solvent in place of extracts. Acarbose at 1mg/mL (in sodium phosphate buffer) was used as standard. Enzyme inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{AbsS}}{\text{AbsE}}\right) \times 100$$

where AbsS is the absorbance of sample and AbsE is the absorbance of enzyme.

Derivatisation for GC Q-TOF MS

Derivatisation was done for the sample in order to improve peak determination and obtain explicit GC Q-TOF MS profiling. Samples were prepared by carefully following the method described by Proestos and Komaitis with some modification[17]. Briefly, 2 mL of the sample (in methanol) was taken in amber vial (initially rinsed with toluene and methanol) and solvent was evaporated off. Afterward, 3 mL of ethyl acetate was added to each, vortexed, and de-humidified with sodium sulphate. In reaction tubes, 100 µL of samples were added to 250 µL of BSTFA (kit, Supelco) and 50 µLof DMSO (to catalyse the reaction). The reaction media was vortexed and kept in an incubator at 60 °C overnight.

GC Q-TOF MS determination

The pre-derivatised samples were injected into a GCMS Agilent system of model 7200 accurate-mass GC Q-TOF MS connected to a 7890A GC system and the detector was quadropole time of flight (Q-TOF) mass spectrometer. The spectrometer was equipped with an Agilent J&W GC column of model- HP-5MS of dimension 30 cm x 0.25 mm x 0.25 mm. It was run following the conditions utilised by Proestos and Komaitis[17]. Temperatures were set for the injector and detector at 280 and 290 °C, respectively. GC was done using splitless mode with 1 min splitless time. The temperature was programmed as follows: from 70 to 135 °C with a 2 °C/min, hold for 10 min, from 135 to 220 °C with a 4 °C/min, hold for 10 min, from 220 to 270 °C with a 3.5 °C/min and then hold for 20 min. A 10 min post run at 70 °C was deemed sufficient for the next injection. The carrier gas flow rate was maintained at 1.9 mL/min[17]. Identification of compounds was done by matching the retention time of the peaks obtained with that of spectral data using Wiley and NIST 14 libraries.

Statistical analysis

The TFC, TPC, and DPPH assays were performed in triplicate and the results are expressed as means±SD using Microsoft Excel. The enzyme analysis was performed in six times replication, It was evaluated by analysis of variance by one-way ANOVA followed by post hoc analysis using Tukey's post t- test and Dunnett's test using IBM SPSS and $p < 0.05$ was regarded as significant.

Results

Extraction is the key preliminary step in many processes in pharmaceutical, food and agricultural industries as well as in scientific studies. Extraction is essentially carried out to obtain the substances of choice or interest from solid, liquid, or gas matrix by employing stressors. Temperature, amount of sample and solvent, time of extraction, pressure (if applied), flow rate of extracting fluid, and a variety of other related parameter have profound effect on the extraction yield, quality of extract, and quantity of bioactive compounds present in the extract. Table 1 depicts the extraction parameters used for the conventional (maceration) and SFE extractions used for this study.

In vitro DPPH, TPC, and TFC assay

Table 2 summarises the results of the three *in-vitro* antioxidant assays (along with antidiabetic assays) employed in the study namely DPPH, TPC, and TFC for both the $EtOH_{conv}$ and SFE extracts. DPPH process

Table 1—Extraction parameters used in conventional and SFE extractions

Parameters	$Ethanol_{conv}$	SFE
Sample	500 g	10 g
Temperature (°C)	On a boiling water bath (100 °C)	60 °C
Solvent (main)	Ethanol (2.5 L)	CO_2 at 4 mL/min
Co-solvent	Not used	Ethanol at 1 mL/min
Time	Soaking followed by heat reflux 4 x 2 h (7 days total)	2 h
Pressure	Atmospheric and Ethanol vapour pressure	60MPa
Yield	34.07 g; 6.814 %	0.507 g; 5.07 %

is the simplest process, wherein the prospective constituent or extract is mixed with DPPH solution and absorbance is recorded after a defined period. DPPH free radical scavenging method offers the first approach for investigating the antioxidant potential of a substance (compound), an extract, or other biological sources by measuring free radical scavenging potential (H- donating ability). The radical scavengers donate hydrogen to the free radicals in turn producing non-toxic species[18-20]. Interestingly, conventional ethanol extract ($EtOH_{conv}$) showed better results in terms of free radical scavenging potential (low IC_{50}) as well as for the presence of total phenolic and flavonoid contents in comparison to supercritical extract.

α-amylase and α-glucosidase enzyme inhibition assay

As stated earlier, α-amylase and α-glucosidase were used to evaluate the anti-diabetic potential of the extracts. The results are given in Table 2. The $EtOH_{conv.}$ extract showed higher α-amylase (30.08±5.22) and α-glucosidase (38.29±2.01) inhibition than the acarbose (28.24±13.66 and 36.93±2.70, respectively), which was the standard and SFE extract (15.67±4.03 and 28.36±2.01, respectively) at the concentration of 1 mg/mL. Results obtained after carrying out an α-glucosidase assay were found to be significant (p <0.05). The conventional extraction method was observed to be able to extract the antidiabetic and antioxidant compounds in appreciable amount through its exhaustive extraction process carried out at high temperature (100 °C).

Table 2—*In vitro* antioxidant and enzyme inhibitory assay results of $EtOH_{conv.}$ and SFE

Assays	$EtOH_{conv}$	SFE	Acarbose
DPPH, IC_{50} (ug/mL)[a]	42.60	186.619	NT
TPC (mg/g)[a]	55.690	20.48	NT
TFC (mg/g)[a]	1.643	1.25	NT
α-amylase (% inhibition at 1 mg/mL conc.)[b]	30.08±5.227	15.67±4.03	28.24±13.664
α-glucosidase (% inhibition at 1 mg/mL conc.)[b*]	38.29±2.518	28.36±2.01	36.93±2.701

a-no. of replicates=3; All results are significant (p <0.05 significant results); b-no. of replicates= 6, assays performed thrice; *results are significant; NT= Not tested

GC Q-TOF MS results

In the present study, GC Q-TOF MS was used to get chemical profiling of both forms of ethanol extracts[17]. To overcome the problem of inability to vapourise high molecular weight compounds like polyphenols and flavonoids, the sample was derivatised using N,O-Bis(trimethylsilyl)trifluoro acetamide (BSTFA). The hits were matched to that of the NIST library. Fig. 1-2 shows the TIC scans of $EtOH_{conv}$ and SFE extracts and table 3 shows the major compounds present in both the extracts along with the pharmacological activities reported in various literatures and PubChem databases for the compounds detected in both the extracts.

A total of 17 compounds were detected with glycine as a major compound in ethanol extract prepared through conventional method. Compounds like 3-phenylpropionic acid, tetradecapentaenoic acid, butanoic acid, and thieno (2, 3-b) quinoline were detected in $EtOH_{conv}$ alone. SFE extracts more compound hits than EtOH agreeing with the fact that SFE is a more sensitive and technologically advanced method as compared to conventional method of reflux. Table 3 shows all the compounds that have been identified from both the extracts via GC Q-TOF MS.

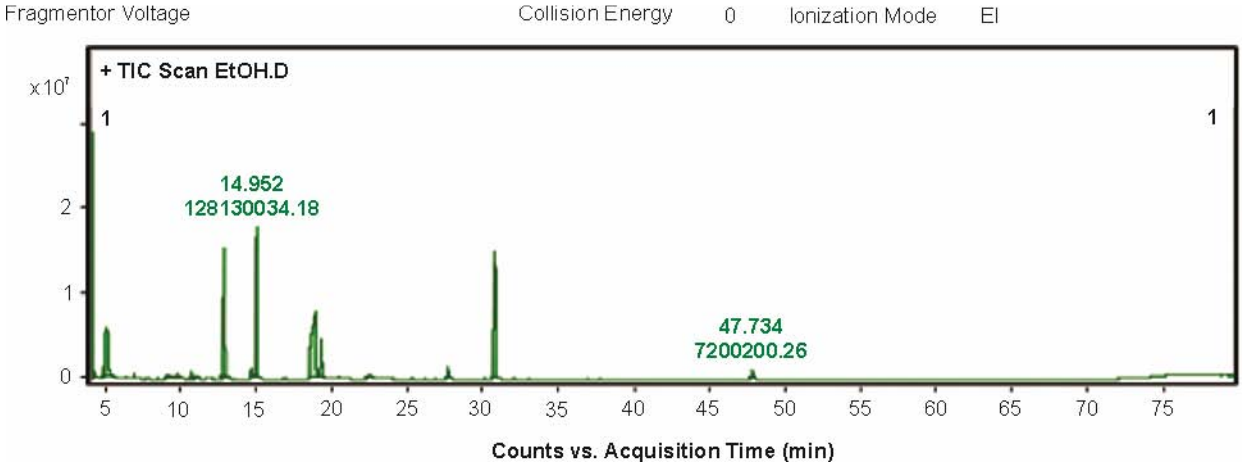

Fig. 1—TIC scan of $EtOH_{conv.}$ extract by GC Q-TOF MS

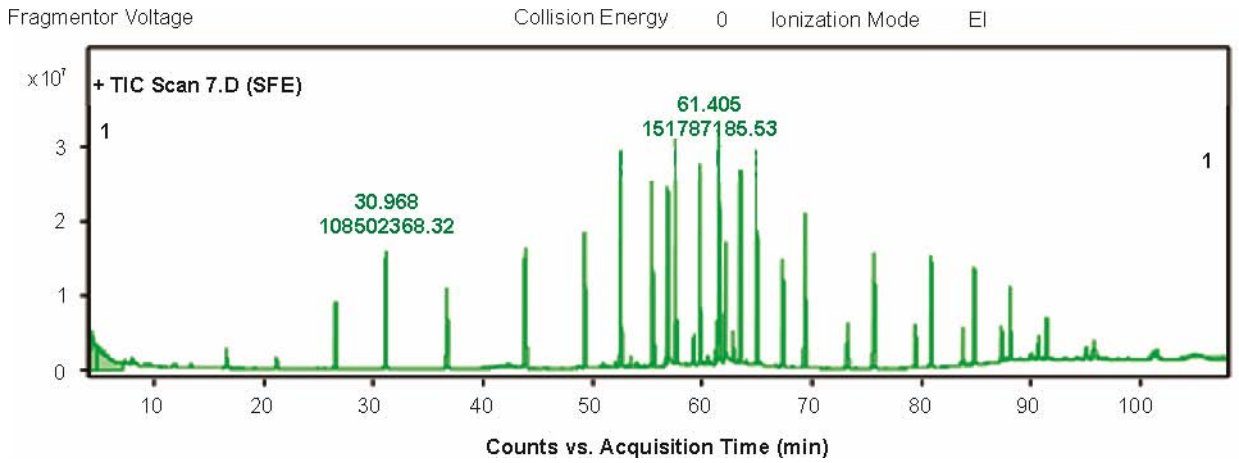

Fig. 2—TIC scan of SFE extract by *GC Q-TOF MS*

Table 3—GCMS chemical profiling with PubChem references and pharmacological activities

Major compounds	Retention time (RT)	Activity/ PubChem or Chemspider, reference no
	Ethanol$_{conv}$ (conventional)	
Glycine	4.09	CID- 750; non-essential amino acid, for fluid irrigation, apoptotic activity , PMID-1960447[21]
N-Aspartic acid (derv.)	4.768	CID- 586809; radical scavenger[22]
(R)-(+)-1- Benzylglycerol	9.725	CID- 98072; anti-cancer/ tumor, anti-oxidant[23]
3-Phenylpropionic acid (Hydrocinnamic acid)	10.948	CID-107, act as an antioxidant to prolong shelf life foods, fungicidal[24]
Thieno(2,3-b) quinolone	14.952	Fungicidal give ref only, anti-cancer[25]
Tetradecapentaenoic acid	19. 221	No database found
Butanoic acid	30.695	CID- 264; anti-diabetic[26], anti-cancer[27]
	SFE extract (non-conventional)	
2', 6'-dihydroxyacetophenone (2-acetyl resorcinol)	11.754	CID 69687, anti-bacterial, antiseptic[28]
Dodecamethyl cyclohexasiloxane	20.996	CID 10911, Used as solvent for industrial uses, in cosmetics and personal care products29
1-Monolinoleoylglycerol	36.546	CID 5283469, prevention or treating athelosclerosis30
2-Methyl-D-glucose	42.25	CID 298225, glycoside
1-Heptatriacotanol	51.207	CID 537071, antioxidant
i-Propyl 12-methyl-tridecanoate	51.951	Ester
Estra-1,3,5(10)-trien-17-beta-ol	56.713	Pentacyclic steroid
Ethyl iso-allocholate	59.09	Chemspider467662, antimicrobial[31]
Cyclopropane butanoic acid	61.038	Free radical scavenger[32]
Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	61.319	Chemspider 4517635, antibacterial, antioxidant[33]
Oleic Acid	61.954	CID 445639, hypotensive34, breast cancer prevention35

Discussion

Extraction method

Interpretation of the data given in table 1 clearly reveals the fact that in spite of slightly higher yield, conventional method consumed higher amount of solvent and took longer time to accomplish the extraction process. According to earlier studies, conventional methods have been reported to produce toxic extracts that might contain high amount of toxic organic solvent leftover that may lead to cancer, other deleterious side effects, and the extracts are not considered readily consumable either[18]. The turn of century has given us better technological outputs and greener technologies like SFE, which has been proven

to be a safe method to produce high quality therapeutic extracts with relatively higher safety profile. SFE method uses less organic solvent and basically uses CO_2 as the main extracting fluid that can be recycled and poses very less impact on the environment[40]. On the other hand, the recovered toxic organic solvent from conventional extraction has to be disposed of, which further poses environmental threat to a great extent[6].

***In vitro* assays**

In terms of antioxidative capacity, conventional extract ($EtOH_{conv}$) showed better antioxidative potential. Conventional extraction (maceration) method is considered a complicated and tedious process and takes a long time for the proper extraction of bioactive components from the plant material as well as it also consumes a large quantity of organic solvent, however, SFE method is completed within a short span of time and doesn't require tedious long hours to prepare reliable bioactive extract. In terms of time factor, it can be deduced that SFE method could have extracted out higher amount of bioactive compounds if the time period for the extraction process had been extended to some extent[7,36]. The flavonoid and phenolic contents in the final extracts are therefore much related and dependent on the extraction methods of choice, time period used, and the solvent properties (polarity) of the solvents[37].

α-amylase and α- glucosidase assay

The digestive enzymes (α-amylase and α-glucosidase) are responsible for post-prandial rise of blood glucose level in a person as these enzymes break down polymeric macromolecules (polysaccharides and disaccharides) into their smaller building blocks (monosaccharide), in order to facilitate their absorption by the body. Keeping in line with the anti-oxidant assays, the ethanol extract obtained through SFE method exhibited less potent inhibitory activity as compared to EtOH extract obtained through conventional method. Nonetheless, its inhibitory activity was found to be in an appreciable range as digestive enzymes inhibitor and it could have shown more potent digestive enzymes inhibitory activity if the extraction period had been increased for a longer period of time at different parameters[38]. SFE extract showed less α-amylase and α-glucosidase inhibitory effect but is considered healthier owing to the fact that SFE process requires low temperature and less amount of food grade ethanol to accomplish extraction process[40]. This could be employed in future to find the best time frame to extract out the highest amount of antioxidants as well as flavonoid and phenolic compounds as digestive enzymes inhibitors (antidiabetic agents) from this weed.

GC Q-TOF MS analysis

The $EtOH_{conv}$ extract in this study showed good free radical scavenging activity accompanied by high phenolic and flavonoid contents. The same extract showed potent digestive enzymes inhibitory activity (i.e. anti-diabetic activity) which could be attributed to the combined or synergistic effects of the free radical scavenging compounds like glycine, butanoic acid and benzyl glycerol. It can be assumed that the GC Q-TOF MS method didn't successfully detect higher molecular weight compounds like flavonoids which were detected through TFC assay. This can be attributed to incomplete derivetisation of the sample or needs for higher detection temperature[17].

The most interesting compound is thieno (2,3-b)quinoline that has been reported to show anti-cancer and fungicidal effects (Table 3). Butanoic acid commonly known as butyric acid was also detected that has been reported to help in energy homeostasis inside the body regulating diabetes[26]. The reported pharmacological properties of such compounds further support the traditional claims and different uses of *M. pudica* in folk remedies as an anti-infective, anti-microbial, anti-cancer, and anti-diabetic agent[36]. The SFE extract showed more interesting bioactive compounds hits than the conventional extract like resorcinol, oleic acid, monolinoleoyl glycerol, ethyl isoallocholate, cyclopropane butanoic acid, heptatriacotanol, etc.

Interestingly, the GC Q-TOF MS result did not reveal the same common compounds in SFE ethanol extract that were detected in $EtOH_{conv}$ extract. In terms of compounds hits, a total of 41 compounds were obtained and some compounds were of great interest from pharmacological effects perspective (Table 3). For instance, resorcinol as a potent antibacterial and antiseptic agent was identified in SFE ethanol extract along with other compounds like 1-monolinoleoylglycerol, 1-heptatriacotanol, ethyl iso-allocholate, cyclopropane butanoic acid and oleic acid, which have not been identified before in this plant. All these compounds are grouped under the category of free radical scavengers and could be responsible for the antidiabetic effect of *M. pudica* through digestive enzymes inhibitory mode of action.

The major compounds in SFE ethanol extract were found to be 1-monolinoleoylglycerol and ethyl iso-allocholate. In term of bioactive compounds, SFE ethanol extract provided some very potent bioactive compounds in a short time frame as well as through the least toxic process. Hence, SFE ethanol extract can later be effectively used to obtain these potent bioactives in a higher yield through further study by adjusting the parameters during extraction process using SFE method[39-40].

Conclusion

The current comparative research work was executed to propose an agricultural waste or weed namely *M. pudica* as an alternative medicinal and useful bioactive source for the management of diabetes. This weed, commonly found in crop and farming lands and often looked upon as a pest, which could in fact be used as potent bioactive herb in the form of antioxidant agent that might be effectively employed as lead or therapeutic source as anti-diabetic, fungicidal, anti-microbial as well as for the management of several diseases equally. Results showed that *M. pudica* is a rich source of antioxidants and can be efficaciously used against diabetes mellitus to control post-prandial high blood sugar. Although, ethanol extract of *M. pudica* obtained through conventional extraction process showed better results in all the assays taken into account but from safety perspectives, SFE could prove to be a better choice as it also indubitably depicted significant digestive enzymes inhibitory effect along with high total phenolic and flavonoid contents and a good source of other potent bioactive compounds namely resorcinol, oleic acid, and ethyl iso-allocholates. Moreover, ethanol as a bio-solvent is considered more acceptable due to its lower toxicity, is easily metabolised inside body, and being a greener fluid in all sense. Hence, results of this study suggest that *M. pudica* could be an effective alternative source for the generation of safe bioactive as future prospects of new drugs.

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Conflict of interest

None to declare.

References

1 Santana-Mèridas O, Gonzàlez-Colema A and Sànchez-Viqoue R, Agricultural residues as source of bioactive natural products, *Phytochem Rev*, 2012, **11**, 447-466.

2 Conde E, Reinoso, B D, Gonzalez-Munoz, M J and Andres Moure, A, Recovery and concentration of antioxidants from industrial effluents and processing streams of underutilized vegetal biomass, *Food Pub Health*, 2013, 3, 69-91.

3 Rout S P, Choudary K A, Kar D M, Das L and Jain A, Plants in traditional medicinal system- Future source of new drugs, *Int J Pharm Pharm Sci*, 2009, **1**(1), 1-23.

4 Ibañez E, Herrero M, Mendiola J A and Castro-Puyana M, Extraction and characterization of bioactive compounds with health benefits from marine resources: macro and micro algae, cyanobacteria, and invertebrates, In: Marine Bioactive Compounds, Springer US, 2012, 55-98.

5 Moure A, Cruz J M, Franco D, Domínguez J M, Sineiro J, Domínguez H, *et al*., Natural antioxidants from residual sources, *Food chem*, 2001, **72**(2), 145-171.

6 Chemat F, Vian, M A and Cravotto G, Green extraction of natural products: Concept and principles, *Int J Mol Sci*, 2012, **13**, 8615-8627.

7 Reverchon E and Marco I D, Supercritical fluid extraction and fractionation of natural matter, *J Supercrit Fluid*, 2006, **38**, 146–166.

8 United States Department of Agriculture (USDA), Weed risk of *Mimosa pudica* (Fabaceae)- Sensitive Plant, Animal and Plant health Inspection Service, Raleigh, 2014, Version 1.

9 Tunna T S, Ahmed Q U, Ahmed Helal A B M and Zaidul I S M, Weeds as alternative useful medicinal source: *Mimosa pudica* L. on *diabetes mellitus* and its complications, *Adv Mater Res*, 2014, **995**, 45-59.

10 Tunna T S, Zaidul I S M, Ahmed Q U, Ghafoor K, Al-Juhaimi F Y, Uddin M S, *et al*., Analyses and profiling of extract and fractions of neglected weed *Mimosa pudica* Linn. traditionally used in Southeast Asia to treat diabetes, *S Afr J Bot*, 2015, **99**, 144-152.

11 Grigonis D, Venskutonis P R, Sivik B, Sandahl M and Eskilsson C S, Comparison of different extraction techniques for isolation of antioxidants from sweet grass (Hierochlo¨e odorata), *J Supercrit Fluid*, 2005, **33**, 223–233.

12 Nickavar B, Kamalinejad M and Izadpanah H, Free radical and food additives *in vitro* free radical scavenging activity of five *Salvia* species, *Pak J Pharm Sci*, 2006, **20**, 291-294.

13 Singleton V L and Rossi J A, Colorimetry of total phenolics with phosphotungstic acid reagents, *Am J Enol Viticul*, 1965, **16**, 144-158.

14 Zhishen J, Tang Mengcheng T and Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chem*, 1999, **64**, 555-559.

15 Worthington V, Alpha amylase, in Worthington enzyme manual, V. Worthington, Ed, 1993 Freehold, NJ, Worthington Biochemical Corporation, 36-41.

16 Apostolidis E, Kwon Y I and Shetty K, Inhibitory potential of herb, fruit, and fungal enriched cheese against key enzymes linked to type 2 diabetes and hypertension, *Innov Food Sci Emerg Tech*, 2007, **8**, 46-54.

17 Proestos, C and Komaitis M, Analysis of naturally occurring phenolic compounds in aromatic plants by RP-HPLC coupled to diode array detector (DAD) and GC-MS after silylation, *Foods*, 2013, **2**, 90-99.
18 Pak-Dek M S, Osman A, Sahib N G, Saari, N, Markom M, Hamid A A, *et al*., Effects of extraction techniques on phenolic components and antioxidant activity of Mengkudu (*Morinda citrifolia* L.) leaf extracts, *J Med Plants Res*, 2011, **5**(20), 5050-5057.
19 Soler-Rivas C, Espin J C and Wichers H J, An easy and fast test to compare total free radical scavenger capacity of food stuffs, *Phytochem Anal*, 2000, **11**, 1-9.
20 Roginsky V and Lissi E A, Review of methods to determine chain-breaking antioxidant activity in food, *Food Chem*, 2005, **92**, 235-254.
21 Zhong Z, Wheeler M D, Li X, Froh M, Schemmer P, Yin M, *et al.*, L-Glycine: a novel antiinflammatory, immunomodulatory, and cytoprotective agent, *Curr Opinion Clin Nutr Metab Care*, 2003, **6**(2), 229-240.
22 Noda Y, Ogata K and Mori A, Antioxidant activities of novel alpha-lipoic acid derivatives: N-(6, 8-dimercaptooctanoyl)-2-aminoethanesulfonate- and N-(6, 8- dimercaptooctanoyl)-L-aspartate-zinc complex, *Res Comm Mol Path Pharmacol*, 2002, **113**, 133-147.
23 Mukai K, Okabe K and Hosose H, Synthesis and stopped flow investigation of antioxidant activity of tocopherols finding of new tocopherol derivatives having the highest antioxidant activity among phenolic antioxidants, *J Org Chem*, 1989, **54**, 557-560.
24 Narayana K J, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y and Krishna P S, Biological activity of phenylpropionic acid isolated from a terrestrial Streptomycetes, *Pol J Microb*, 2007, **56**, 191-197.
25 Rechfeld F, Gruber P, Kirchmair J, Boehler M, Hauser N, Hechenberger G, *et al.*, Thienoquinolines asnovel disruptors of the PKCε/RACK2 protein–protein interaction, *J Med Chem*, 2014, **57**, 3235–3246.
26 Tilg H and Moschen A R, Microbiota and diabetes: An evolving relationship, *Gut*, 2014, **63**(9), 1513-1521.
27 Zimmerman M A, Singh N, Martin P M, Thangaraju M, Ganapathy V, Waller J L *et al*., Butyratesuppresses colonic inflammation through HDAC1-dependent Fas-up regulation and Fas-mediated apoptosis of T cells, *Am J Physio Gastroint Liver Physiol*, 2012, **302**, 1405–1415.
28 Boer J and Jemec G B, Resorcinol peels as a possible self-treatment of painful nodules in hidradenitis suppurativa, *Clinic Exp Dermatol*, 2010, **35**, 36–40.
29 EPA, Office of Pollution Prevention and Toxics, High Production Volume (HPV) Challenge Program, 2009, available from the Database Query page at: http://www.epa.gov/hpv/pubs/general/opptsrch.htm
30 Lee W S, Kim M J, Beck Y I, Park Y D and Jeong T S, Lp-PLA2 inhibitory activities of fat glycerols isolated from *Saururus chinensis* roots, *Bioorg med Chem lett*, 2005, **15**, 3573-3575.
31 Singariya P, Kumar P and Mourya K K, Isolation of some new steroids and evaluation of bioactivity of *Cenchrus ciliaris*, *Int J Res Pharm Sci*, 2012, **3**, 678-684.
32 Lide D R and Milne G W A, Handbook of data on common organic compounds, 1995, CRC Press, 1, 278.
33 Baharudin M K A, Shafida A, Hamid S A and Susanti D, Chemical composition and antibacterial activity of essential oils from three aromatic plants of the zingiberaceae family in Malaysia, *J Phys Sci*, 2015, **26**, 71–81.
34 Teres S, Barcelo-Coblijn G, Benet M, Alvarez R, Bressani R, Halver J E *et al*., Oleicacid content is responsible for the reduction in blood pressure induced by olive oil, *Proc Nat Acad Sci*, 2008, **105**, 13811–13816.
35 Pala V, Krogh V, Muti P, Chajes V, Riboli E, Micheli A *et al*., Erythrocyte Membrane Fatty Acids and Subsequent Breast Cancer: A Prospective Italian Study", *J Nation Cancer Instit*, 2001, **93**, 1088–95.
36 Tamilarasi T and Ananthi T, Phytochemical analysis and anti-microbial activity of *Mimosa pudica* L., *Res J Chem Sci*, 2012, **2**, 72-74.
37 Apaya K L and Chichioco-Hernandaz C L, Xanthine oxidase inhibition of selected Phillipine medicinal plants, *J Med Plant Res*, 2011, **5**, 289-292.
38 Bandar H, Hijazi A, Rammal H, Hachem A, Saad Z and Badran B, Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioca*, *Am J Phytomed Clin Therap*, 2013, **1**, 507-513.
39 Huie C W, A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants, *Anal Bioanal Chem*, 2002, **373**, 23-30.
40 Smith R M, Before the Injection-modern methods of sample preparation for separation techniques, *J Chromatogr A*, 2003, **1000**, 3-27.