Comparative antioxidant and antidiabetic effects of *Syzygium polyanthum* leaf and *Momordica charantia* fruit extracts

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Abstract: The study was aimed to perform aqueous extraction of two plants using different extraction methods, and evaluate their antioxidant and antidiabetic potential. Plant materials were extracted by maceration, soxhlet, sonication and fresh juice methods to produce aqueous extracts. *In vitro* antioxidant DPPH (1,1-diphenyl-2- picrylhydrazyl) and FRAP (Ferric reducing antioxidant power), antidiabetic α–amylase and α–glucosidase enzyme inhibitory assays were carried out on the extracts. Extracts of *Syzygium polyanthum* demonstrated better free radical scavenging and antidiabetic activity than *Momordica charantia*. It was observed that the % inhibition of DPPH by fresh juice of *S. polyanthum* was 64.93 similar to quercetin 69.21 ($p>0.05$). Its FRAP value (69.05) was significantly ($p<0.05$) higher than Quercetin (63.27). Its fresh juice also demonstrated significant inhibitory actions ($p<0.05$) against α-amylase (92.21%) and α–glucosidase (96.06%) than acarbose. It is concluded that extracts had varied results due to differences in their chemical composition as noticed in LC-MS. The fresh juice of *S. polyanthum* has superior *in vitro* antioxidant and antidiabetic activities. Therefore, intake of exogenous antioxidants in the form of fresh juices of some herbs can help the body to scavenge free radicals and exert hyperglycaemic control in post prandial hyperglycaemia.

Keywords: *Syzygium polyanthum*, *Momordica charantia*, Antioxidant, Antidiabetic, extraction.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease or disorder with multiple aetiologies, is characterized by high blood sugar levels accompanied by impaired metabolism of carbohydrates, lipids, and proteins as a result of insufficiency of insulin function. Its common symptoms include thirst, polydipsia, polyphagia, weight loss, polyuria, itching, and weakness. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Environmental factors, diet and free radicals also play a role in the development of diabetes. Among all, free radicals are the most concerned, free radicals are atoms, molecules, or ions with unpaired electrons with open shell configuration, free radicals may have positive, negative or neutral charge, and many free radicals are unstable and highly reactive. The formation of free radicals can be attributed to cellular metabolism, cellular respiration, exposure to ionizing radiation, smoking, herbicides, pesticides and fried foods. When free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress will occur and cause increased tissue injury. Therefore, free radicals or reactive oxygen species are implicated as possible underlying pathogenic mechanisms in the progression of cardiovascular diseases including ischemic heart disease, atherosclerosis, cardiac arrhythmia, hypertension and diabetes (Singh *et al.*, 2014).

Generally diabetes mellitus can be handled in several ways such as dietary adjustments, regular exercise, the use of oral antidiabetic drugs like sulfonylureas, biguanides and as well as insulin injections. However, the drugs in the market have considerable side effects and are expensive. Therefore, many patients in developing countries are relied on alternative treatments, such as complimentary alternative medicine with natural herbs. *Gymnema sylvestre*, green tea (*Camellia sinensis*), and *Trigonella foenum-graecum* are a few that have substantial evidence in glycaemic control (Shahidi and Ambigaipalan, 2015). The use of traditional medicine is based on the knowledge gained from inheritance and hence patient compliance is high for such remedies. Effects of herbal medicine is influenced by the form of presentation of herbal drugs that we consume (Kalichevsky *et al.*, 1995). A study conducted by Faller & Fialho (2009) revealed that vegetables such as Potato, Carrot, Onion, Broccoli, and White cabbage upon subjecting to boiling, microwaving, and steaming can exhibit significant differences in antioxidant properties (Faller *et al.*, 2009). Therefore, the selection of optimum best extraction methods is often necessary to get the maximum efficacy with high amounts of the active compounds in the herbal products.

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Syzygium polyanthum (S. polyanthum), commonly referred as "Indonesian bay leaf" of Myrtaceae family is reported to have anti-inflammatory, antipyretic and detoxificant properties against various poisons. The leaves of the plant are fragrant due to the presence of citral, eugenol in its volatile oil. In addition, several phenolic compounds such as tannins, flavonoids and phenolic acids were reported in the plant. A preparation called "Jamu" is a traditional herbal medicine that has been consumed for many centuries in the Indonesian community to maintain good health and to treat diseases and usually prepared by decoction (Elfahmi *et al*., 2014). There are several plants used in "Jamu" based on the required therapeutic response, the plants that are used in Jamu for antidiabetic use are *S. polyanthum* leaf and *Momardica charantia* (*M. charantia)* fruit. *M. charantia* also known as bitter gourd or bitter melon belongs to Cucurbitaceae family and is the most frequently used plant based antidiabetic medicine. The plant is rich in phytoconstituents like glycosides, saponins, phenolics, fixed oils, resins and alkaloids. Several studies have reported antidiabetic, anticancer, anti-inflammatory, antiviral and hypocholesterolemic activities for the fruits of the plant. Both *M. charantia* and *S. polyanthum* are rich in polyphenolic flavonoids, tannins and alkaloids as mentioned in the literature. In Japan and China a green tea (*Camellia sinensis*), a folklore medicine is popular, the chemo preventive effects of green tea are well established, benefits probably attributed in particular to the catechin polyphenolic components. Green tea is normally consumed as a brewed tea, though most chemo preventive applications have used a concentrated extract. Green tea catechins may act on multiple pathways to prevent cancer, including oxidative stress, elimination of carcinogens, and inhibition of enzymes (Shahidi and Ambigaipalan, 2015). Therefore, dietary intake of these herbs can play an important role in the body's antioxidant defence which most likely mediate their beneficial health effects by scavenging free radicals responsible for oxidative stress associated with cancer, diabetes, hypertension, cardiovascular and neurodegenerative diseases, longevity and aging (Hui *et al.,* 2015; Robert, 2014; Hugel *et al.,* 2016). To get maximum benefit from these herbs proper method of extraction should be optimized before formulating herbal products to acquire health benefits.

In view of the above, a study evaluating the effect of *S. polyanthum* leaf extract and *M. charantia* fruit extract produced by different extraction methods such as maceration, sonication, soxhlet, fresh juice and their antioxidant and antidiabetic potential *in vitro* was examined. It is known that choosing right food, controlled sugar levels and the exogenous antioxidants can benefit in chronic ailments such as diabetes since good food is an important part to lead a healthy lifestyle. The study is expected to give an input on the free radical scavenging abilities of the selected vegetable plants and the role of extraction in their antioxidant and antidiabetic efficiency.

MATERIALS AND METHODS

Chemicals and reagents

Phloroglucinol and glycerol were purchased from Merck (Germany), HPLC grade methanol, 1,1-Diphenyl-2-picryl-hydrazyl, quercetin, 2,4,6-tripyridyl-s-triazine, $FeCl_3$, $FeSO_4$, glacial acetic acid, sodium acetate trihydrate, potato starch, sodium chloride, sodium phosphate monobasic, 3,5-dinitrosalicylic acid, potassium tartrate tetrahydrate, sodium hydroxide, sodium carbonate, 4-Nitrophenyl-β-D-glucopyranoside, enzyme α-amylase and enzyme α-glucosidase were procured from Sigma-aldrich (St Louis, MO, USA), potassium phosphate monobasic and acarbose 95% were obtained from Acros organic (USA). All other chemicals and reagents were of analytical quality grade and were used as received.

Plant materials

Leaves of *S. polyanthum* were collected from Selangor, Malaysia in the month of June, 2014 from local terrains. However, the fruits of *M. charantia* were purchased from the traditional market in Pulau Pinang, Malaysia in the same period. Both the plants were authenticated by a Taxonomist Dr. Sugumaran Manickam at Rimba Ilmu facility available within University Malaya campus, Kuala Lumpur, Malaysia. A herbarium specimen was deposited and Voucher specimens of the samples *S. polyanthum* (KLU 49084) and *M. charantia* (KLU 49083) were kept in the Rimba Ilmu. Plant materials were washed separately with fresh water to remove superficial dirt, processed to exclude inner pulp and seeds of the *M. charantia* fruit and were dried in oven for 2 days at temperature below 50°C. The dried materials were pulverized into powder by commercial blender (National, Malaysia) and the materials were stored in airtight polypropylene containers at room temperature (25°C) for future use.

Microscopic evaluation of plant samples

Freshly collected leaves of *S. polyanthum* were evaluated for their transverse section of the lamina and midrib region to evaluate microscopic characters of the leaf as shown in fig. 1. Similarly freshly collected fruits of *M. charantia* were evaluated as shown in fig. 2, a thin section of the rind of the fruit of *M. charantia* was taken to evaluate microscopic characters of the fruit for evaluation of its identity. A thin section was taken by placing a piece of leaf midrib of *S. polyanthum* and a piece of fruit rind of *M. charantia* in potato block to ensure manual sectioning. The section was immediately treated with phloroglucinol and concentrated HCl for identification of lignin and tissue differentiation. Glycerol was added as mounting solution and as humectant before placing a coverslip for microscopical examination. Finely powdered samples were treated similarly and both powder and transverse sections were observed under light microscope at a magnifications of 4x, 10x and 40x using Olympus CH30

(Olympus, Japan) microscope, photographs were taken using a digital camera and Scanning electron microscope (SEM) by Quanta FEG 450, USA. The transverse sections and powder microscopic characters of *S. polyanthum* leaf and *M. charantia* fruit were as shown in fig. 1 and fig. 2.

Extraction of plant samples

Extraction by maceration

The maceration method was adopted from Montanez *et al*. (2014). Briefly, dried coarse fruit powder of *M. charantia* and dried fine leaf powder of *S. polyanthum* (250g) was mixed with 5000mL and 1500mL of water respectively, both powders were agitated using mechanical stirrer (1500rpm) for 30 minutes to ensure uniform powder and solvent mix. The beaker was covered with aluminium foil and was kept for 3 days under refrigeration at 5-8^{o}C. The macerated mixture was filtered through a muslin cloth and subsequently filtered by vacuum filtration. The final filtrate was freeze dried using Labconco freezone freeze dryer, USA.

Fresh juice

About 1Kg of fresh *M. charantia* fruit devoid of seeds and fresh *S. polyanthum* leaves were cut into small pieces. The chopped pieces were homogenized in a commercial blender (National, Malaysia) with water to *M. charantia* (1:2) and water to *S. polyanthum* (1:4) ratios. The homogenized mixture was then stirred at 1500 rpm for 15 min. The fresh juice was filtered through a muslin cloth and subsequently filtered by vacuum filtration. The final filtrate was freeze dried (Kumar *et al*., 2009).

Extraction by sonication

A 250g dried coarse fruit powder of *M. charantia* and dried fine powder of *S. polyanthum* leaf were mixed with 5000mL and 1500mL of water respectively, both powders were stirred at 1500 rpm for 15 min. A bath sonicator (Fisher scientific FB15057, USA) was used for sonication of the sample for 30 minutes at a constant frequency of 37 kHz at a temperature of 30^{o}C. The sonicated mixture was filtered through a muslin cloth and subsequently filtered by vacuum filtration. The final filtrate was freeze dried.

Soxhlet extraction

The dried coarse fruit powder of *M. charantia* and dried fine leaf powder of *S. polyanthum* weighing 250g was transferred in a "thimble" made of cellulose placed in the chamber of the Soxhlet apparatus. About 5000mL of water was used as a solvent in the flask. The condensed vapours of solvent drip into the thimble containing the sample and ensure hot percolation of solvent to produce an efficient extraction. This process was carried out until the residue completely exhausted. The soxhlet extract was filtered through vacuum filtration. The final filtrate was freeze dried.

LC-MS phytochemical screening of extracts

LC-MS screening was carried out as per the modified method described by Terpinc *et al*. (2016). LC-MS instrument used was an Agilent 6550 iFunnel Q-TOF LC-MS series. The sample was analysed by the LC-MS instrument with a 10µL sample injection (1mg/10mL in Methanol, HPLC grade Merck, Germany). The chemical profile of the sample was derived using a C-18 column at a flow rate of 0.2mL/min. The solvent gradient of phase A consisted of 0.1% formic acid in water, phase B consisted of 0.1 % formic acid in methanol: 19% A, 81% B from 0 to 10 min, 21% A, 79% B from 10 to 15min, 28% A, 72% B from 15 to 35min. Positive ion electrospray ionization (ESI) was used for the detection of the analytes without solvent splitting.

Antioxidant activities of the extracts

DPPH assay

Antioxidant activity test using DPPH or 1,1-Diphenyl-2-picryl-hydrazyl (Sigma, Germany) was carried out as per the modified method described by Brand-Williams *et al*. (1995). The reaction mixture was prepared in a 96-well micro plate (Solid clear F-bottom, Greiner Bio One, Austria) adding 20µL of sample (1mg/mL extract) and 120µL of 100µM DPPH in methanol and incubated in dark at 25°C for 20min. The absorbance of the solutions was measured using UV-Visible spectrophotometer (Infinite M 200 Tecan, Switzerland) at 517 nm. Free radical scavenging activity of the samples was estimated by the colour change from deep purple to yellow and decrease in the absorbance value in comparison to the blank is an indication of antioxidant activity. The radical scavenging activity percentage (RSA %) of the samples was evaluated with (Eq. 1). The mixture of methanol (20 µL) and DPPH (120µL) served as blank, quercetin (Sigma-aldrich, St Louis, MO, USA) mixture of DMSO (20µL) and DPPH radical solution (120µL) served as control.

$$\text{RSA\%} = \frac{\text{Ao} - \text{As}}{\text{A0}} \times 100 \qquad \text{(Eq. 1)}$$

FRAP assay

The FRAP assay or Ferric reducing antioxidant power was performed as per the previous method described by Udayaprakash *et al*. (2015). A fresh working solution was prepared by mixing 25 mL acetate buffer 300mM, pH 3.6, 2.5mL TPTZ 10mM (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 2.5mL $FeCl_3 \cdot 6H_2O$ 20mM. The reagent was warmed at 37°C before use, the assay was carried out in a 96-well micro plate (Solid clear F-bottom, Greiner Bio One, Austria) with 10µL (1mg/mL) of individual extract solution with 300 µL of FRAP solution incubated for 30 min in dark. Absorbance of each well was measured at 593 nm using UV-Visible spectrophotometer (Infinite M 200 Tecan, Switzerland). The experiment for all the extracts was repeated in triplicate. The percentage ferric (Fe^{3+}) reduction to ferrous (Fe^{2+}) was calculated by

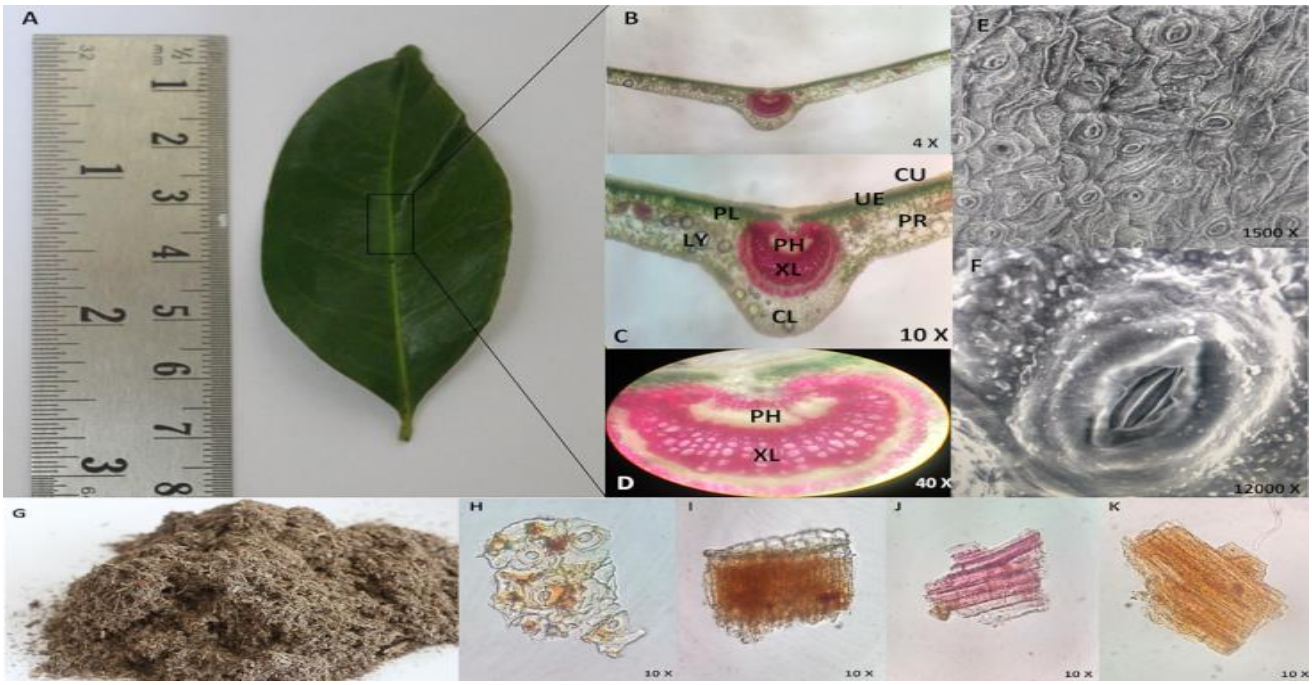

Fig. 1: A. *Syzygium polyanthum* fresh leaf; B. Transverse section of leaf at 4x magnification; C. Cuticle (CU), Upper epidermis (UE), Palisade (PL), Lysigenous oil cells (LY), Parenchyma (PR), Xylem (XL), Phloem (PL), Collenchyma (CL); D. Xylem and Phloem at 40x; E & F. Paracytic stomata observed under Scanning Electron Microscope under 1500x and 12000x magnification; G. *Syzygium polyanthum* leaf powder; H. Paracytic Stomata; I. Mesophyll; J. Xylem; K. Sclerenchyma.

$FeSO_4$ standard curve (R^2 = 0.9959) between 200 to 1000 µM using the equation 2.

FRAP value = Abs (sample +FRAP reagent) - Abs (FRAP reagent)

$$\%\,\text{FRAP} = \frac{\text{FRAP Value of Sample}}{\text{FRAP Value of } FeSO_4\,7H_2O} \times 100 \quad \text{(Eq.2)}$$

Antidiabetic enzyme inhibitory activities of the extracts

α-amylase inhibitory activity

The α-amylase assay was evaluated using the method of Loizzo *et al*. (2007) with few modifications. Briefly, 20 µL of aqueous sample solution (1mg/mL extract) or standard acarbose 95% (Acros organic, USA) was mixed with 50µL of phosphate buffer solution of α-amylase enzyme (Porcine Pancreas Amylase; 5mg/10mL; 10 Unit/ mg; Sigma-aldrich, St Louis, MO, USA) and was incubated at 37°C for 10min. To this mixture 100 µL of starch solution (1% w/v of potato starch [Sigma-aldrich, St Louis, MO, USA] in pH 6.9 phosphate buffer prepared by mixing 20mM monobasic sodium phosphate and 6.7 mM of sodium chloride in 50mL and heated at 65°C for 15 min), was added with an incubation of 10 min. at 37 °C. The reaction was terminated by adding 100µL of 3, 5-dinitrosalicylic acid 96mM (prepared by mixing 15g of sodium potassium tartrate tetra hydrate in 10ml of 2M NaOH and 0.5mg 3, 5-dinitrosalicylic acid solution) and was further incubated in water bath for 10min. The colorimetric reagent was prepared by mixing a sodium potassium tartrate solution and 0.5mg 3, 5-dinitrosalicylic acid solution. Control and sample extracts were added with starch solution and left to react with α-amylase solution under an alkaline condition at 25°C. The reaction was measured over 3 min. The generation of maltose was quantified by the reduction of 3, 5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. This reaction is detectable at 540 nm by using spectrophotometer UV-Vis (Infinite M 200 Tecan, Switzerland) the assay was carried out in 96-well micro plates (Solid clear F-bottom, Greiner Bio One, Austria). The percentage inhibition was calculated using the equation 3.

$$\%\,\text{Inhibition} = \frac{\text{A blank - A sample}}{\text{A blank}} \times 100 \quad \text{(Eq. 3)}$$

α-glucosidase inhibitory activity

The α-glucosidase assay was evaluated using the method of Li *et al*. (2007) with few modifications. The assay was determined in 96-well micro plates (Solid clear F-bottom, Greiner Bio One, Austria). Briefly, 40µL of aqueous sample solution (1 mg/mL extract) or standard acarbose 95% (Acros organic, USA) was mixed with 100µL of phosphate buffer solution of α-glucosidase enzyme (*Saccharomyces cerevisiae*; 2.2mg/10mL; 10Unit/mg; Sigma-aldrich, St Louis, MO, USA) and was incubated at 37°C for 10min. To assay mixture 50 µL of 4-Nitrophenyl β-D-glucopyranoside (PNPG) substrate solution (5mM of PNPG [Sigma-aldrich, St Louis, MO, USA] in pH 6.9 phosphate buffer prepared by mixing 67mM monobasic

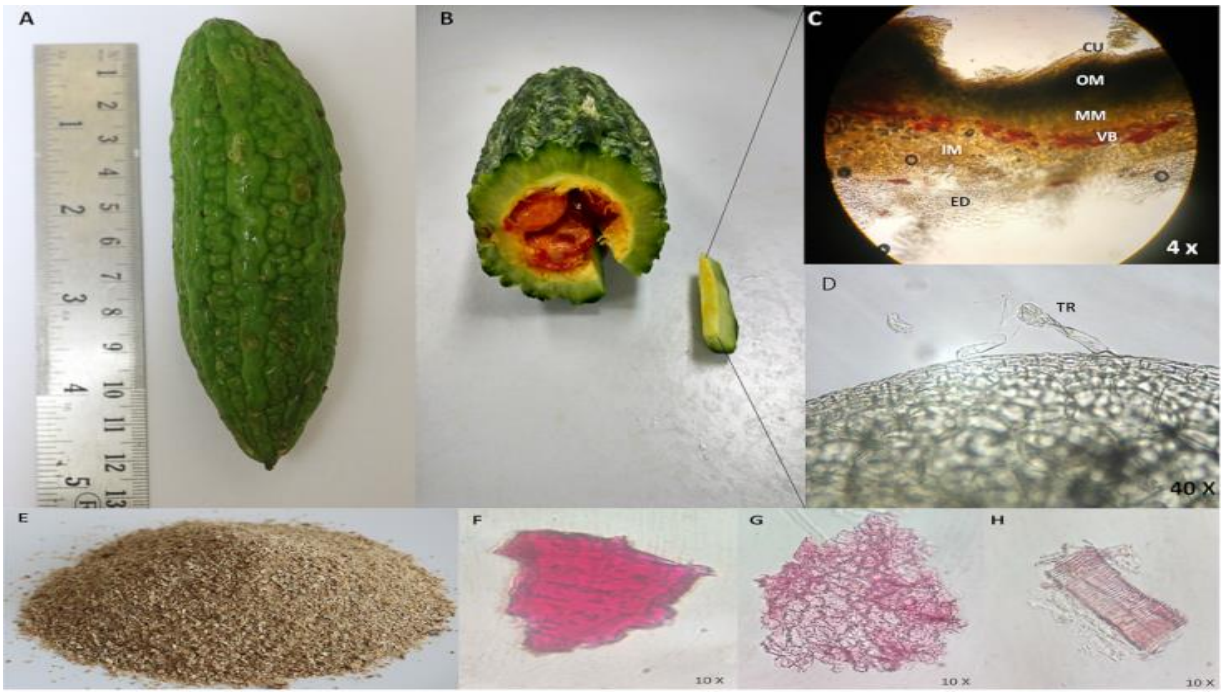

Fig. 2: A. *Momordica charantia* fresh fruit; B. Piece of fruit sample; C. Cuticle (CU), Outer mesocarp (OM), Middle mesocarp (MM), Vascular bundle (VB), Inner mesocarp (IM); Endocarp (ED) D. Glandular trichome (TR). E. *Momordica charantia* fruit powder; F. Stone cells; G. Sclerenchyma; H. Lignified xylem

potassium phosphate), was added with an incubation of 10 min. at 37°C. The reaction was stopped by adding 80µL of 100mM Na_2CO_3. This reaction was measured on spectrophotometer UV-Vis (Infinite M 200 Tecan, Switzerland) at 405 nm. The percentage of inhibition was calculated using the equation 4.

$$\% \text{ Inhibition} = \frac{\text{A blank - A sample}}{\text{A blank}} \times 100 \qquad \text{(Eq. 4)}$$

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS 22 statistical package (IBM Software, USA). The data were analysed by one way analysis of variance (ANOVA) followed by Duncan test. $p<0.05$ is considered as statistically significant. All the results were expressed as mean ± SD for triplicate determinations.

RESULTS

S. polyanthum grows wild in the forests and mountains, in the garden and in waste lands. This tree can be found in lowlands up to an altitude of 1400m above sea level. Leaves are simple oval-shaped with elliptical tapered tip, base, fin shaped, upper surface is dark green, light green coloured lower surface, 5-15 cm long, 3-8 cm wide, fragrant (Soh and Parnell, 2011). *S. polyanthum* showed the presence of cuticle outside of bifacial leaf, upper epidermis was thin-walled, palisade layer contained 1-2 layers of columnar shaped cells, 8-16 layered loose spongy parenchyma of mesophyll and had spherical lysigenous oil cells, the midrib showed the lignified vascular bundles, xylem and phloem, collenchyma had a thick walled and tightly arranged cells. The microscopic characteristics of *S. polyanthum* powder showed lignified xylem vessels with spiral thickenings, sclerenchyma, mesophyll and characteristic paracytic type of stomata, each stoma was surrounded by two subsidiary cells which are parallel to the longitudinal axis of the pore and guard cells as observed under SEM (fig. 1).

The fruit of *M. charantia* was elongated, ribbed, 8-30cm long, hard seeds with size 8-13mm, elongated flat shaped with irregular grooves (Gupta *et al*., 2011). *M. charantia* fruit showed epidermis with epicarp covered with a thick striated cuticle and has prominent irregular large ridges and tapering outgrowths which are extensions of the pericarp. Epidermis consists of relatively small parenchymal layer rich in chlorophyll. The epidermis was also found with uniseriate multicellular glandular trichomes. Sub-epidermal tissue composed of several layers of round to oval cells enclosing chloroplasts and lignified sclerenchymatous bands of cells. Aqueous mucilaginous mesocarp consisted of large oval parenchymatous cells without chlorophyll occasionally with lignified sclerenchymatous cells. It was constituted by several layers of almost colourless isodiametric parenchyma cells. *M. charantia* fruit powder showed fragments of lignified stone cells, lignified xylem and sclerenchyma (Aswar and Kuchekar, 2012) (fig. 2).

Table 1: List of identified phytoconstituents in the fruit aqueous extracts of *Momordica charantia* by LC-MS

No	Compound name	Elemental composition	Mass (g/mol)	m/z	RT(min.)
1	5S-HETE di-endoperoxide	$C_{20}H_{34}O_8$	402.22	441.18	1.19^{η}
2	Tyr Arg Ser	$C_{18}H_{28}N_6O_6$	402.22	425.21	1.20^{η}
3	11-amino-undecanoic acid	$C_{11}H_{23}NO_2$	201.17	202.18	4.16^{η}
4	Nonactin	$C_{40}H_{64}O_{12}$	736.43	605.36	4.26^{η}
5	2-Amino-3-methyl-1-butanol	$C_5H_{13}NO$	103.09	104.10	4.26^{η}; 4.23^{Ω}; 4.18^{λ}
6	Adenine	$C_5H_5N_5$	135.05	136.06	4.59^{η};4.70^{Ψ}
7	Anthraquinone	$C_{14}H_8O_2$	208.05	209.06	4.69^{η};5.11^{Ω}
8	Cis-1,2-Dihydroxy-1,2-dihydrodibenzothiophene	$C_{12}H_{10}O_2S$	218.04	219.04	4.70^{η}; 4.74^{Ω}; 4.82^{λ}
9	Allo-inositol	$C_6H_{12}O_6$	180.06	203.05	4.82^{η};4.82^{Ω};4.79^{λ}
10	1,3,7-Trimethyluric acid	$C_8H_{10}N_4O_3$	210.07	233.06	4.83^{η}
11	Sphinganine	$C_{16}H_{35}NO_2$	273.26	274.27	4.86^{η};4.88^{Ω};4.93^{Ψ}
12	Phytosphingosine	$C_{18}H_{39}NO_3$	317.29	318.29	4.87^{η};4.85^{Ω}; 4.89^{λ};4.90^{Ψ}
13	y-Glu-Cys	$C_8H_{14}N_2O_5S$	250.06	251.07	5.07^{η}
14	3-propylmalic acid	$C_7H_{12}O_5$	176.06	199.05	5.14^{η};5.12^{Ω}
15	δ-Valerolactam	C_5H_9NO	99.06	100.07	5.24^{η};5.23^{Ω};5.25^{Ψ}
16	a-Methyl-3,4-dihydroxyphenylpropionic	$C_{10}H_{12}O_4$	196.07	219.06	5.36^{η}; 5.31^{λ}
17	DiallylTrisulfide	$C_6H_{10}S_3$	177.99	179.00	5.77^{η}
18	Burseran	$C_{22}H_{26}O_6$	386.17	409.16	6.33^{η};6.33^{Ω};6.28^{λ}
19	Apiole	$C_{12}H_{14}O_4$	222.08	245.07	6.49^{η};6.49^{Ω};6.54^{Ψ}
20	Estra-1,3,5(10)-triene-3,6alpha,17beta-triol triacetate	$C_{24}H_{30}O_6$	414.20	437.19	7.28^{η}; 7.27^{Ω}; 7.37^{Ψ}
21	Catechin 3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate)	$C_{22}H_{20}O_9$	428.10	451.10	7.44^{η}; 7.41^{Ω}; 7.32^{λ}
22	Apigenin 7-(3”-acetyl-6”-E-p-coumaroylglucoside)	$C_{32}H_{28}O_{13}$	620.15	643.14	11.00^{η}
23	Proanthocyanidin A2	$C_{30}H_{24}O_{12}$	576.12	599.11	12.9^{η};13^{Ω};12.51^{λ};13.7^{Ψ}
24	Elaidamide	$C_{18}H_{35}NO$	281.27	304.26	9.20^{Ω};9.19^{λ}; 8.02^{Ψ}
25	p-Aminobenzoic acid	$C_7H_7NO_2$	137.04	138.05	4.56^{Ω}; 4.77^{Ψ}
26	Alpha-D-Mannoheptulopyranose	$C_7H_{14}O_7$	210.07	233.06	4.84^{Ω}; 4.84^{λ}; 4.89^{Ψ}
27	1-Deoxy-D-xylulose	$C_5H_{10}O_4$	134.05	157.04	5.03^{Ω}
28	Tert-Butylbicyclophosphorothionat	$C_8H_{15}O_3PS$	222.04	219.03	5.77^{Ω}
29	Soyasaponin III	$C_{42}H_{68}O_{14}$	796.45	819.44	5.81^{Ω}
30	9S-hydroxy-12R,13S-epoxy-10E,15Z-octadecadienoic acid	$C_{18}H_{30}O_4$	310.21	333.20	7.06^{Ω}
31	Eplerenone	$C_{24}H_{30}O_6$	414.20	437.19	7.27^{Ω};7.37^{Ψ}
32	Linoleamide	$C_{18}H_{33}NO$	279.25	302.24	0.59^{λ}
33	24-Nor-5β-cholane-3α,12α,22,23-tetrol	$C_{23}H_{40}O_4$	380.29	381.29	3.24^{λ}
34	Palmitic amide	$C_{16}H_{33}NO$	255.25	278.24	4.56^{λ}; 3.36^{Ψ}
35	Exserohilone	$C_{20}H_{22}N_2O_6S_2$	450.09	451.10	7.32^{λ};7.53^{Ψ}
36	14,14,14-Trifluoro-11Z-tetradecenyl acetate	$C_{16}H_{27}F_3O_2$	308.19	309.20	0.3^{Ψ}
37	gamma-Pentachlorocyclohexene	$C_6H_5Cl_5$	251.88	274.87	4.40^{Ψ}
38	Cycloleucine	$C_6H_{11}NO_2$	129.07	130.08	4.52^{Ψ}
39	5-Aminopentanoic acid	$C_5H_{11}NO_2$	117.07	118.08	4.76^{Ψ}
40	Derrone	$C_{20}H_{16}O_5$	336.10	337.10	4.79^{Ψ}

Continue…

41	Sophoracoumestan A	$C_{20}H_{14}O_5$	334.08	335.09	4.79^{Ψ}
42	Erythrinin A	$C_{20}H_{16}O_4$	320.10	321.11	4.83^{Ψ}
43	Neuraminic acid	$C_9H_{17}NO_8$	267.09	268.10	4.83^{Ψ}
44	L-Galactose	$C_6H_{12}O_6$	180.06	203.05	4.88^{Ψ}
45	Benserazide	$C_{10}H_{15}N_3O_5$	257.10	258.10	4.89^{Ψ}
46	Corynebactin	$C_{39}H_{42}N_6O_{18}$	882.25	905.24	4.91^{Ψ}
47	Quebrachitol	$C_7H_{14}O_6$	194.07	217.06	4.95^{Ψ}
48	Xestoaminol C	$C_{14}H_{31}NO$	229.24	230.24	4.95^{Ψ}
49	Linamarin	$C_{10}H_{17}NO_6$	247.10	270.09	4.95^{Ψ}
50	(2S,3S)-2,3-Dihydro-2,3-dihydroxybenzoate	$C_7H_8O_4$	156.04	157.04	5.05^{Ψ}
51	p-Acetaminobenzoic acid	$C_9H_9NO_3$	179.05	180.06	5.07^{Ψ}
52	(+)-Eudesmin	$C_{22}H_{26}O_6$	386.17	409.16	6.39^{Ψ}

Table 2: List of identified phytoconstituents in the leaf aqueous extracts of *Syzygium polyanthum* by LC-MS

No	Compound name	Elemental composition	Mass (g/mol)	m/z	RT(min.)
1	Stearamide	$C_{18}H_{37}NO$	283.28	306.27	3.38^{η}
2	Palmitic amide	$C_{16}H_{33}NO$	255.25	278.24	$3.67^{\eta};3.59^{\Omega}$
3	2-Amino-3-methyl-1-butanol	$C_5H_{13}NO$	103.09	104.10	$4.18^{\eta};4.41^{\lambda};4.18^{\Psi}$
4	5-Aminopentanoic acid	$C_5H_{11}NO_2$	117.07	140.06	$4.62^{\eta};4.73^{\lambda}$
5	Theobromine	$C_7H_8N_4O_2$	180.06	203.05	$4.8^{\eta};4.83^{\lambda};4.80^{\Psi}$
6	Sphinganine	$C_{16}H_{35}NO_2$	273.26	274.27	$4.84^{\eta};4.82^{\Omega};$ $4.86^{\lambda};4.83^{\Psi}$
7	1-Deoxy-D-xylulose	$C_5H_{10}O_4$	134.05	157.04	$5.00^{\eta};5.00^{\Omega};5.08^{\lambda}$
8	Karanjin	$C_{18}H_{12}O_4$	292.07	293.08	$5.00^{\eta};5.00^{\Omega}$
9	3-propylmalic acid	$C_7H_{12}O_5$	176.06	199.05	$5.10^{\eta};5.10^{\Omega}$
10	a-Methyl-3,4-dihydroxyphenylpropionic acid	$C_{10}H_{12}O_4$	196.07	219.06	5.3^{η} ;$5.32^{\Omega};5.40^{\lambda}$
11	(+)-Eudesmin	$C_{22}H_{26}O_6$	386.17	409.16	$6.25^{\eta};6.26^{\Psi}$
12	Adifoline	$C_{22}H_{20}N_2O_7$	424.12	425.13	$6.25^{\eta};6.27^{\Omega}$
13	Apiole	$C_{12}H_{14}O_4$	222.08	245.07	$6.43^{\eta};6.44^{\Omega};$ $6.52^{\lambda};6.44^{\Psi}$
14	Exserohilone	$C_{20}H_{22}N_2O_6S_2$	450.09	451.10	$7.29^{\eta};7.29^{\Omega};7.30^{\Psi}$
15	Elaidamide	$C_{18}H_{35}NO$	281.27	304.26	7.75^{η}
16	Proanthocyanidin A2	$C_{30}H_{24}O_{12}$	576.12	599.11	$12.34^{\eta};12.37^{\Omega};$ 13.60^{λ}
17	Allo-Inositol	$C_6H_{12}O_6$	180.06	203.05	4.80^{Ω}
18	Tiaprofenic acid	$C_{14}H_{12}O_3S$	260.05	261.05	4.89^{Ω}
19	Anthraquinone	$C_{14}H_8O_2$	208.05	209.06	5.07^{Ω}
20	Burseran	$C_{22}H_{26}O_6$	386.17	425.13	$6.27^{\eta};6.36^{\lambda}$
21	2,8-Dihydroxyadenine	$C_5H_5N_5O_2$	167.04	168.05	3.99^{λ}
22	Linamarin	$C_{10}H_{17}NO_6$	247.10	248.11	4.20^{λ}
23	Perindoprilat	$C_{17}H_{28}N_2O_5$	340.20	341.20	4.32^{λ}
24	D-Glucoheptose	$C_7H_{14}O_7$	210.07	329.31	4.84^{λ}
25	Phytosphingosine	$C_{18}H_{39}NO_3$	317.29	318.30	4.86^{λ}

Continue…

26	Phenazine-1,6-dicarboxylic acid	$C_{14}H_8N_2O_4$	268.04	269.05	5.11^{λ}
27	δ-Valerolactam	C_5H_9NO	99.06	100.07	5.27^{λ}
28	Diallyl Trisulfide	$C_6H_{10}S_3$	177.99	219.03	5.81^{λ}
29	Catechin 3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate)	$C_{22}H_{20}O_9$	428.11	451.10	7.50^{λ}
30	Apigenin 7-(3"acetyl-6"-E-p-coumaroylglucoside)	$C_{32}H_{28}O_{13}$	620.15	643.14	11.37^{λ}
31	3,4-Dihydroxybenxylamine	$C_7H_9NO_2$	139.06	140.07	4.64^{Ψ}
32	Gln Cys Asp	$C_{12}H_{20}N_4O_7S$	364.10	365.11	4.68^{Ψ}
33	Disialyllactose	$C_{34}H_{56}N_2O_{27}$	924.30	947.29	5.02^{Ψ}
34	Aminofurantoin	$C_8H_8N_4O_3$	208.05	109.06	5.08^{Ψ}
35	Syringic acid	$C_9H_{10}O_5$	198.05	199.06	5.11^{Ψ}
36	Salvianolic acid	$C_{26}H_{22}O_{10}$	494.11	495.12	7.10^{Ψ}

η=Maceration; Ψ=Soxhlet; λ= Sonication; Ω= Fresh juice

The order of yield value obtained from various aqueous extracts of *M. charantia* were Sonication >Soxhlet > Maceration >Fresh Juice. The best method was sonication extraction with maximum percentage yield of 26.37% (65.93g). Heat involved soxhlet extraction method also significantly showed higher yield of 24.25 % (60.63g) next to sonication. The yields of maceration and fresh juices were 12.98% (32.44g) and 3.06% (30.63g) respectively. On the contrary the order of yield value obtained from various extracts of *S. polyanthum* were Fresh Juice >Soxhlet >Sonication >Maceration. The best method for *S. polyanthum* with maximum percentage yield was fresh juice with 10.07% (100.73g). The yields of maceration, sonication and soxhlet in *S. polyanthum* were 7.44% (18.6g), 8.22% (20.54g) and 8.7% (21.75g) respectively.

In addition, the aqueous extracts of both *S. polyanthum* and *M. charantia* were subjected to LC-MS analysis. The mass spectrum, fragmentation pattern of the phytoconstituents, retention time, *m/z* ions and their metabolites was investigated using LC-QTOF. The constituents identified for both plants were summarized in table 1 and table 2. Results revealed that as many as 52 phytoconstituents are present in *M. charantia* extracts combined. In comparison the *S. polyanthum* extracts had lesser, 36 identified phytoconstituents. Some of the constituents are specific to the extraction method used.

As shown in the fig. 3, the DPPH scavenging potential for the extracts of *M. charantia* differed based on the source of the extract, 19.76% inhibition was observed with soxhlet sample. The fresh juice showed 14.19 whereas 9.16, 8.53% for maceration and sonication extracts respectively. The positive control had 69.21 % inhibition. The difference in activities of extracts obtained from different methods was highly significant (p<0.001). Among the four extracts maceration and sonication were statistically similar as the difference in DPPH activity between them was insignificant (p>0.05). However, soxhlet extract was significantly different from fresh juice as well as maceration and sonication (p<0.05). Therefore, the selection of extraction method has affected the DPPH scavenging ability of the extracts. Among the *S. polyanthum* extracts, the % inhibition for maceration, fresh juice, sonication and soxhlet extracts were 64.18, 58.03, 64.93 and 63.15 % respectively. It was observed that *S. polyanthum* irrespective of method of extraction there was no major difference among their activities and were close to the standard, Quercetin (69.21%). The DPPH free radical scavenging, inhibitory activities of *M. charantia* extracts were relatively insignificant when compared to *S. polyanthum* extracts.

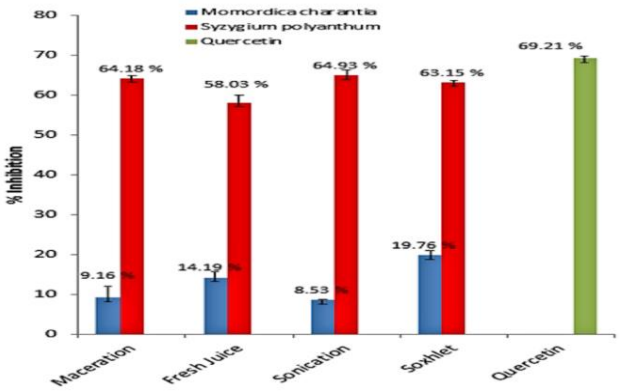

Fig. 3: DPPH radical scavenging activity aqueous extracts of two selected plants, data was presented as percent DPPH inhibition, mean ± SD (n=3)

FRAP assay was carried out for extracts of both the plants. As shown in fig. 4 the FRAP inhibitory values of *M. charantia* were in between 3.12 to 4.19%. The positive control had 63.27 % inhibitory value. The difference in activities of extracts was insignificant (p>0.05). This suggests that *M. charantia* extracts, despite difference in method of extraction were similar in showing FRAP activity. The Ferric Reducing Antioxidant Power for extracts of *S. polyanthum* was 17.56% (Maceration),

69.05% (Fresh Juice), 29.24% (Sonication) and 28.21% (Soxhlet). Interestingly the fresh juice of *S. polyanthum* was better than the Quercetin (63.27%) and it was statistically significant (p<0.05). This can be ascribed to the ability of the compounds detected in *S. polyanthum* to donate electrons for their antioxidant power. This suggests that the method of extraction had lot of difference in extracting the phytoconstituents.

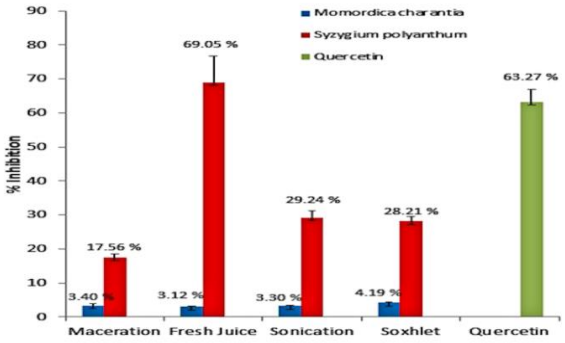

Fig. 4: Ferric Reducing Antioxidant Power of different aqueous extracts of two selected plants, data was presented as percent FRAP inhibition, mean ± SD (n=3)

The fresh juice of *M. charantia* exhibited highest α-amylase inhibition of 61.24% followed by sonication (57.06%), maceration (51.27%) and soxhlet (43.2%). Unlike DPPH, FRAP results of *M. charantia* its enzyme inhibitory activity was better though different from acarbose (p<0.05). A noticeable difference was observed between the four extracts of *S. polyanthum*, and the activities were significantly different (p<0.001). The fresh juice of *S. polyanthum* showed 92.21%, significantly (p< 0.05) higher than acarbose (88.51%) as in fig. 5. All four different extracts of *M. charantia* possessed statistically similar α–glucosidase inhibitory activity (p>0.05). The

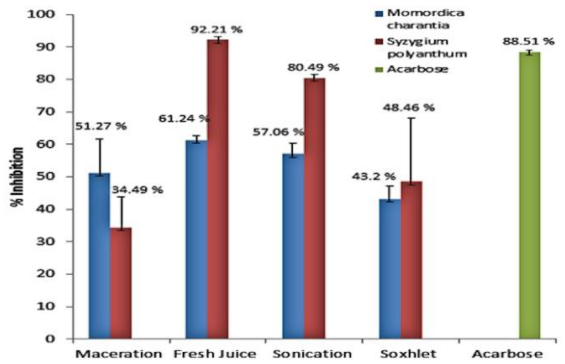

Fig. 5: α-amylase inhibitory effects of aqueous extracts of two selected plants, data was presented as percent α-amylase inhibition, mean ± SD (n=3)

values of the extracts ranged between 16.47 to 21.77% and the standard acarbose had 32.22% (fig. 6). The extract produced by cold maceration showed highest 21.77% inhibition in comparison to others. In contrary, the results of α-glucosidase inhibitory effects of *S. polyanthum* were distinct between 16.57 to 96.06% and acarbose with 32.22 % only. The fresh juice of *S. polyanthum* exhibited excellent α-glucosidase inhibitory activity than the rest with 96.06 % inhibition (fig. 6).

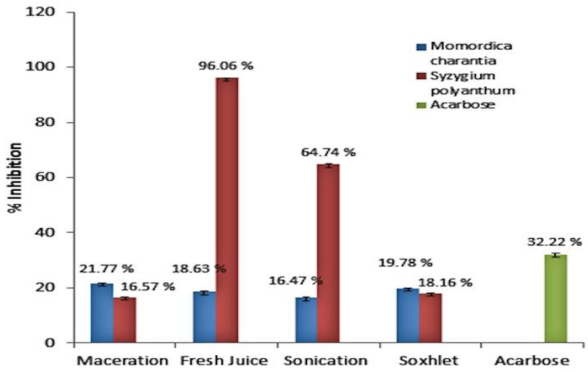

Fig. 6: α-glucosidase inhibitory effects of aqueous extracts of two selected plants, data was presented as percent α-glucosidase inhibition, mean ± SD (n=3)

DISCUSSION

Free radicals are generated either due to metabolism within the biological systems or externally acquired, severely affect human health leading to several diseases, such as aging, cardiovascular, neurogenerative diseases, diabetes and cancer**.** Free radicals and oxidative damage was identified as one of the major causes of diabetes (Yokozawa *et al*., 2007). Nutraceuticals, vitamins, vegetables, fruits and beverages can avoid or limit oxidative damage (Wu and Ng, 2008). Polyphenols are rich in food and green vegetables, hence can protect oxidative damage by scavenging free radicals. Tocopherols, Vitamin C are natural antioxidants in plants capable of scavenging lipid peroxyl radicals and quenching of oxygen free radicals (Munne-bosch and Alegre, 2002). Hence, herbs with rich phenolic contents are often included in herbal medicine to provide health benefits by scavenging free radicals. The term herbal medicine refers to herbs, herbal materials, herbal preparations and finished herbal products, which contain active plant parts, either as powders or as extracts, or as their combination.

In the current study free radical scavenging ability of two plant extracts obtained via different extraction methods was evaluated. The two plant samples were screened for authenticity and identity prior to extraction. One of the common problems associated with herbs is inconsistency and heterogeneity in the quality. The quality of herbal raw materials fluctuates greatly due to geographical location, soil environment, mode of collection, climatic conditions, habit and habitats. Therefore, standardization of herbal

products has been strongly recommended to overcome disparity among them. Important attributes of standardization procedure include proper authenticity and purity of the samples selected for the herbal products. Authenticity of the sample can be done either by macroscopy or microscopy or by chemical evaluation or by genotypic analysis. Macroscopy can be applied to distinguish the desired herb from its common adulterant by observing the herbaceous, woody or succulent nature of the plant. While the leaf (shape, size, margin etc.), flower (simple or compound, inflorescence, arrangement of carpels and stamens, sex of the flowers etc.) and fruit (type, dehiscence or indehiscence etc.) morphologies will be utilised to discriminate from adulterants. On the contrary microscopy is helpful to analyse the microscopical characters employing microscopes to visualize type of trichomes, epidermis, oil glands, vascular tissues, cells and cell inclusions. Therefore detailed morphological and microscopical characters of the two selected plant species were carried out to confirm the authenticity and identity. The macro and microscopical characters of the two selected species were in correlation to the existing available literature.

Extraction of plant materials is critical in isolation and purification of phytoconstituents. In the current study water was selected as a solvent of choice primarily due its association to life and dependence of humans in daily routine. Among the *M. charantia* extracts ultrasound extraction produced more yield by cavitation phenomena that created surface disruption during sonication which resulted in surface peeling, erosion and particle breakdown offering increased yield (Chemat *et al.,* 2017). Further, the effect of sonication on the media and effects of micro mixing, macro-turbulence due to cavitation bubbles created during the process might have augmented the yield. Soxhlet extraction also exhibited good yield by enhanced diffusiveness of the solvent into the material due to decreased viscosity and solubility induced desorption of the compounds to contribute to the effect (Chemat *et al.,* 2017). In contrary to the *M. charantia* extraction *S. polyanthum* showed varied yields. Large 1 kg fresh sample of *S. polyanthum* selected for fresh juice could have been contributed greatly to its high yield. The reason for selection of 1kg for fresh juice was based on the fact that 1kg of fresh sample is equivalent to 250 g of dry plant material used in other extracts. Since fresh samples were subjected to shear forces in a blender that enhanced solvent penetration and extraction, gave better yields. In addition relative differences in the hardness of the plant materials, particle size and differences in polar soluble components offered varied yields in the two plants.

Several phytoconstituents were identified upon LC-MS profiling of the plant extracts based on their mass fragmentation patterns in comparison to the standard literature data. The identified compounds were classified as phenolics (Catechin 3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate, Apigenin 7-(3”-acetyl-6”-E-p-coumaroylglucoside, Proanthocyanidin A2, Apiole), Lignans (Burseran), Isoflavones (Derrone, Sophoracoumestan A), Sphingolipids (Spinganine, Phytosphingosine), Saponins (Soyasaponin), Steroids (Eplerenone), Glycosides (Linamarin), Quinones (Anthraquinone), Aminoacids (Arginine, Tyrosine, cysteine), Fatty acids and phenolic acids (11-amino-undecanoic acid, 9S-hydroxy-12R,13S-epoxy-10E,15Z-octadecadienoic acid, p-Aminobenzoic acid), Sugars (Allo-inositol) and Hormones ((+)-Eudesmin). In comparison to *M. charantia*, the *S. polyanthum* extracts had lesser, 36 phytoconstituents than its counterpart which showed 52. Phenolic compounds (Catechin 3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylate, Apigenin 7-(3”-acetyl-6”-E-p-coumaroylglucoside, Proanthocyanidin A2, Apiole), Lignans (Burseran), Sphingolipids (Spinganine, Phytosphingosine), Glycosides (Linamarin), Quinones (Anthraquinone) and Hormones ((+)-Eudesmin) were common in both plants. However, few characteristic phytoconstituents noticed in *S. polyanthum* were an alkaloid (Adifoline), a flavonol (Karanjin), a lactam (Valerolactam), a phytotoxin (Exserohilone), polyhydroxy sugars (1-Deoxy-D-xylulose, D-Glucoheptose, Disialyllactose), abundant fatty acid derivatives (Theobromine, stearamide, palmitic amide, salvianolic acid), and phenolic acids (5-Aminopentanoic acid, 3-propylmalic acid, a-Methyl-3,4-dihydroxyphenylpropionic acid, tiaprofenic acid, phenazine-1,6-dicarboxylic acid, syringic acid).

Free radicals including reactive oxygen species (ROS) and reactive nitrogen species are generated in our body by various endogenous systems, exposure to different physiochemical conditions, or pathological states, and have been implicated in the pathogenesis of many diseases (Cheng *et al.,* 2003; Arya *et al.,* 2012; Slater, 1984). As a defence against oxidative damage, the body normally maintains a variety of mechanisms to prevent such damage while allowing the use of oxygen for normal functions. Such “antioxidant protection” derives from sources both inside the body (endogenous) and outside the body (exogenous). Endogenous antioxidants include molecules and enzymes. Exogenous antioxidants are derived usually from food, food derived antioxidants and phytoconstituents of plants. Therefore, the exogenous antitoxidant capabilities of the two selected plant extracts was tested using DPPH method. The DPPH method is based on the principle of decolourization of DPPH solution by the sample and intensity of measurement of the absorbance at 517 nm. DPPH free radical reacts with compounds capable of donating hydrogen and a significant reduction in the absorbance of reaction mixture is considered as significant free radical (DPPH) scavenging effect of the sample (Krishnaiah *et al*., 2011).

Earlier reports on fruit constituents of *M. charantia* and *M. cochinchinensis* have identified flavonoids, coumarins, anthraquinones, anthocyanins and phenolic acids responsible for the antioxidant activity (Daniel *et al.*, 2014; Nagarani *et al.*, 2014). DPPH scavenging ability was very low for *M. charantia* extracts it was ranged between 8.53-19.76 %. DPPH reacts with hydrogen radical or an electron to become stable molecule (El-Maati *et al.*, 2016). All the extracts of *S. polyanthum* showed consistent DPPH scavenging effect with inhibition ranging from 58.03-64.93%. The results of *S. polyanthum* extracts indicated that they had high ability to donate these hydrogen radicals and electrons to DPPH. According to Kiokias *et al*. (2008) phenolic compounds have the ability to donate hydrogen radicals to DPPH and thereby scavenge these radicals. In order to donate its hydrogen atom by a phenolic compound its reduction potential should be lower than the reduction potential of the free radical (Shahidi and Ambigaipalan, 2015). Though, many of the identified phytoconstituents were similar in *M. charantia* and in *S. polyanthum* extracts there were considerable differences in DPPH scavenging. Both had phenolic compounds but the extracts of *S. polyanthum* had higher reduction potential to donate hydrogen than *M. charantia.*

Another antioxidant study namely FRAP assay was tested on the extracts to know their exogenous antioxidant abilities. FRAP assay gives the reducing power of the sample by transferring an electron to the targeted molecule to convert from ferric (Fe^{3+}) tripyridyltriazine complex to formation of ferrous (Fe^{2+}) tripyridyltriazine (Kubola and Siriamornpun, 2008; Benzie and Strain, 1996) and subsequent colour change from green to blue. It has been noticed that FRAP activity of plant extracts was due to their phenolic content (El-Maati *et al.*, 2016). Common phenolic compounds in *M. charantia* are catechin, gallic acid, gentisic acid, chlorogenic acid and epicatechin (Horax *et al.*, 2010; Kubola and Siriamornpun, 2008). *M. charantia* extracts showed negligible FRAP activity with 3.12-4.19% inhibition.

The fresh juice of *S. polyanthum* was considered very significant in showing FRAP activity among the four extracts with 69.05 % inhibition. The LC-MS profiling of the *S. polyanthum* extract has identified electron rich amides, theobromine, stearamide, palmitic amide and antioxidant phenolic acids (5-Aminopentanoic acid, 3-propylmalic acid, a-Methyl-3,4-dihydroxy phenyl propionic acid, tiaprofenic acid, phenazine-1,6-dicarboxylic acid, syringic acid). The macerated extract of *S. polyanthum* had the least FRAP activity with 17.56 % inhibition. This can be attributed to prolonged duration of extraction (3 days) that might have caused the hydrolysis of phytoconstituents in the extract reducing its antioxidant power. In addition the presence of water soluble theobromine, a methylxanthine alkaloid, its reduction upon storage due to fermentation might also be contributed to the effect (Benzie and Strain, 1996; Niemenak *et al*., 2006; Maleyki and Ismail, 2010; Smit *et al*., 2004).

As mentioned earlier *S. polyanthum* leaf extracts and *M. charantia* fruit extracts have been traditionally used by local Indonesian folk for diabetes in the name of "Jamu" preparation. Therefore, current study was carried out to evaluate α-amylase and α-glucosidase inhibitory properties of their extracts. Fresh juice of *M. charantia* showed significant α-amylase inhibitory activity with 61.24% inhibition. Constituents of *M. charantia* such as flavonoids, charantin, glycosides and steroids have been reported for the hypoglycaemic activity (Kumar *et al.*, 2009). Some of these compounds were detected in qualitative LC-MS analysis of its extracts except charantin. Charantin is insoluble either in highly polar solvent (water) or highly non-polar solvent (benzene) instead has high solubility in chloroform and dichloromethane. *M. charantia* has been well known for its antidiabetic effects*;* some researchers found that its fresh fruit juice can restore key antioxidant enzymes, superoxide dismutase, xanthin oxidase and catalase in diabetic patients (Tayyab and Lal, 2013; Lin *et al.*, 2012). Fresh juice of *S. polyanthum* showed highly significant (p<0.01) α-amylase inhibitory activity with 92.21% inhibition. It is worth mentioning that fresh juice, of *S. polyanthum* consistently showed better results in DPPH, FRAP and also in α-amylase inhibitory studies**.** Phytochemical investigation by Widyawati *et al*. (2015) on the *S. polyanthum* revealed the presence of flavonoids, glycosides, alkaloids and tannins from the leaves. This is in correlation to our findings by LC-MS analysis.

The α-glucosidase inhibitory effect of *M. charantia* was poor with values ranging from 16.47 to 21.77%. Despite poor α-glucosidase inhibitory effect of *M. charantia* its antidiabetic effects were well demonstrated in literature, the activity was ascribed mostly to the enhanced glucose uptake by liver, inhibiting essential enzymes of gluconeogenesis (Glucose-6-phosphatase and Fructose-1,6-biphosphatase), increased glucose oxidation, enhanced glucose uptake by cells, promoting the number of insulin producing β-cells and potentiating insulin release (Chuang *et al.*, 2006; Mahomoodally *et al.*, 2004; Mahomoodally *et al.*, 2007). Thus its antidiabetic effect cannot be ruled out as it is believed to act by multiple mechanisms. Few researchers have even considered *M. charantia* as herbal remedy for type-II diabetes. The α-glucosidase inhibitory effects of *S. polyanthum* were 16.57 to 96.06%. The fresh juice of *S. polyanthum* exhibited excellent 96.06% inhibition significant (p< 0.001) than acarbose (32.22%). Therefore, the fresh juice of *S. polyanthum* due to its ability to inhibit α-amylase and α-glucosidase can significantly lower post-prandial hyperglycaemia.

CONCLUSION

Choice of diet and dietary products is critical in human health; foods and vegetables rich in antioxidant phytoconstituents such as polyphenolic flavonoids, phenolic acids, tannins and vitamins are beneficial and reduce the risk associated with oxidative stress and degenerative diseases. Our study revealed that, method of extraction has a significant effect on the number of constituents in an extract and significant differences in pharmacological effects can be found based on the method employed for extraction. Two selected plants, *M. charantia* and *S. polyanthum* were successfully extracted by four different extraction methods and their results were established. The identified phytoconstituents were in accordance with the existing literature, antioxidant tests such as DPPH, FRAP and enzymatic antidiabetic tests coincided with the chemical composition. The study has reiterated the necessity for standardization and optimization of herbal products and traditional medicine. The study favours natural dietary supplements rich as exogenous antioxidants, in amounts sufficient to prevent complications of oxidative stress. Therefore, the fresh juice of *S. polyanthum* can be antioxidant and antidiabetic both as alone or in combination with *M. charantia* suitable for inclusion as a health supplement and can reduce postprandial glucose levels in diabetic patients.

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REFERENCES

Arya A, Nyamathulla S, Noordin MI and Mustafa AM (2012). Antioxidant and Hypoglycemic activities of leaf extracts of three popular terminalia species. *E-Jour. Chem.,* **9**(2): 883-892.

Aswar PB and Kuchekar BS (2012). Phytochemical, microscopic, antidiabetic, biochemical and histopathological evaluation of Momordica charantia fruits. *Int. J. Pharm. Pharm. Sci.,* **4**(1): 325-331.

Benzie IFF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.,* **239**(1): 70-76.

Brand-Williams W, Cuvelier ME and Berset C (1995). Use of free radical method to evaluate antioxidant activity. *Leb. und-Technologie.,* **28**: 25-30.

Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS and Abert-Vian M (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.,* **34**: 540-560.

Cheng HY, Lin TC, Yu KH, Yang CM and Lin CC (2003). Antioxidant and free radical scavanging activities of Terminalia chabula. *Pharm. Soc. Japan.,* **26**(9): 1331-1335.

Chuang CY, Hsu C, Chao CY, Wein YS, Kuo YH and Huang CJ (2006). Fractionation and identification of 9c, 11t, 13t-conjugated linolenic acid as an activator of PPAR alpha in bitter gourd (*Momordica charantia* L.). *J. Biomed. Sci.,* **13**(6): 763-772.

Current microscopy contributions to advances in science and technology, Méndez-Vilas, A (ed.), 1st edition, Vol. 2, Formatex research center, Badajoz, Spain. pp.704-710.

Daniel P, Supe U and Roymon MG (2014). A review on Phytochemical analysis of Momordica charantia. *Int. J. Adv. Pharm. Biol. Chem.,* **3**(1): 214-220.

Elfahmi, Woerdenbag JH and Kayser O (2014). Jamu: Indonesia Traditional Herbal Medicine Towards Rational Phytopharmacological Use. *J. Herb Med.,* **4**(2): 1-23.

El-Maati MFA, Mahgoub SA, Labib SM, Al-Gaby AMA and Ramadan MF (2016). Phenolic Extracts of Clove (*Syzygium aromaticum*) with Novel Antioxidant and Antibacterial Activities. *Eur. J. Integr. Med.,* **8**(4): 494-504.

Faller ALK and Fialho E (2009). The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Res. Int.,* **42 (1)**: 210–215.

Gupta M, Sharma S, Gautam AK and Bhadauria, R (2011). Momordica charantia linn. (Karela): Nature's silent healer. *Int. J. Pharm. Sci. Rev. Res.,* **11** (1): 32-37

Horax R, Hettiarachchy N and Chen P (2010). Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of bitter melons (*Momordica charantia*) harvested at three maturity stages (Immature, Mature, and Ripe). *J. Agric. Food Chem.,* **58**(7): 4428-4433.

Hügel HM, Jackson N, May B, Zhang AL and Xue CC (2016). Polyphenol protection and treatment of hypertension. *Phytomedicine*, **23**: 220-231.

Hui C, Yixi X and Xiaoqing C (2015). Type 2 diabetes diminishes the benefits of dietary antioxidants: Evidence from the different free radical scavenging potential. *Food Chem.,* **186**: 106-112.

Kalichevsky MT, Knorr D and Lillford PJ (1995). Potential food applications of high-pressure effects on ice-water transitions. *Trends Food Sci. Technol.,* **6**(8): 253-259.

Kiokias S, Varzakas T and Oreopoulou V (2008). *In vitro* activity of Vitamins, Flavonoids, and Natural Phenolic Antioxidants against the Oxidative Deterioration of

Oil-Based Systems. *Crit. Rev. Food Sci. Nutr.,* **48**(1): 78-93.

Krishnaiah D, Sarbatly R and Nithyanandam R (2011). A Review of the Antioxidant Potential of Medicinal Plant Species. *Food Bioprod. Process.,* **89** (3): 217-233.

Kubola J and Siriamornpun S (2008). Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts in vitro. *Food Chem.,* **110**(4): 881-890.

Kumar R, Balaji S, Um TS and Sehgal PK (2009). Fruit extracts of Momordica charantia potentiate glucose uptake and up-regulate Glut-4, PPARγ and PI3K. *J. Ethnopharmacol.,* **126**(3): 533-537.

Li T, Liu J, Zhang X and Ji G (2007). Antidiabetic activity of lipophilic (-)-epigallocatechin-3-gallate derivative under its role of α-glucosidase inhibition. *Biomed Pharmacother.,* **61**(1): 91-96.

Lin ZY, Liua X, Yang F and Yu YQ (2012). Structural characterization and identification of five triterpenoid saponins isolated from Momordica cochinchinensis extracts by liquid chromatography/tandem mass spectrometry. *Int. J. Mass Spectrom.,* **328**: 43-46.

Loizzo MR, Saab AM, Statti GA and Menichini F (2007). Composition and α-amylase inhibitory effect of essential oils from Cedrus libani. *Fitoterapia,* **78**(4): 323–326.

Mahomoodally MF, Fakim AG and Subratty AH (2004). *Momordica charantia* extracts inhibit uptake of monosaccharide and amino acid across rat everted gut sacs *in vitro*. *Biol. Pharm. Bull.,* **27**(2): 216-218.

Maleyki MJA and Ismail A (2010). Antioxidant Properties of Cocoa Powder. *J. Food Biochem.,* **34** (1): 111-128.

Montanez GR, Sanchez JAR, Santoyo MC, Velázquez-De la cruz GV, Ramirez de leon JA and Ochana AN (2014). Evaluation of extraction methods for preparative scale obtention of mangiferin and lupeol from mango peels (*Mangifera indica* L.). *Food Chem.,* **159**: 267-272.

Munne-bosch S and Alegre L (2002). The function of tocopherols and tocotrienols in plants. *Crit. Rev. Plant Sci.,* **21**(1): 31-57.

Nagarani G, Abirami A and Siddhuraju P (2014). Food prospects and nutraceutical attributes of Momordica species: A potential tropical bioresources A review. *Food Sci. Hum. Wellness.,* **3**(3-4): 117-126.

Niemenak N, Rohsius C, Elwers S, Ndoumou DO and Lieberei R (2006). Comparative study of different cocoa (*Theobroma cacao* L.) clones in terms of their phenolics and anthocyanins contents. *J. Food Compos. Anal.,* **19** (6-7): 612-619.

Robert L (2014). Role of free radicals and xanthine oxidase. *Pathologie Biologie*, **62**(2): 61-66.

Shahidi F and Ambigaipalan P (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects - A review. *J. Funct. Foods.,* **18**: 820-897.

Singh R, Devi S and Gollen R (2014). Role of free radical in atherosclerosis, diabetes and dyslipidemia: larger-than-life. *Diabetes Metab. Res. Rev.,* **32**(30): 13-23.

Slater TF (1984). Free-radical mechanisms in tissue injury. *Biochem. J.,* **222**(1): 1-15.

Smit HJ, Gaffan EA and Rogers PJ (2004). Methylxanthines Are the Psycho-Pharmacologically Active Constituents of Chocolate. *Psychopharmacol.,* **176** (3-4): 412-419.

Soh WK and Parnell J (2011). Comparative leaf anatomy and phylogeny of Syzygium Gaertn. *Plant Syst. Evol.,* **297** (1-2): 1-32.

Tayyab F and Lal SS (2013). Antidiabetic, hypolipidemic and antioxidant activity of *Momordica charantia* on Type-II diabetic patient in Allahabad India. *Int. J. Pharma. Bio. Sci.,* **4**(4): 932-940.

Terpinc P, Cigic B, Polak T, Hribar J and Pozrl T (2016). LC-MS analysis of phenolic compounds and antioxidant activity of buckwheat at different stages of malting. *Food Chem.,* **210**: 9-17.

Udayaprakash NK, Ranjithkumar M, Deepa S, Sripriya N, Al-Arfaj AA and Bhuvaneswari S (2015). Antioxidant, free radical scavenging and GC-MS composition of Cinnamomum iners Reinw. ex Blume. *Ind. Crops. Prod.,* **69**: 175-179.

Widyawati T, Yusof NA, Asmawi MZ and Ahmad M (2015). Antihyperglycemic Effect of Methanol Extract of Syzygiucpolyanthum (Wight.) Leaf in Streptozotocin-Induced Diabetic Rats. *Nutrients*, **7**(9): 7764-7780.

Wu SJ and Ng LT (2008). Antioxidant and free radical scavenging activities of wild bitter melon (Momordica charantia Linn. var. abbreviata Ser.) in Taiwan. *LWT - Food Sci. Technol.,* **41**(2): 323-330.

Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC and Juneja LR (2007). Amla (*Emblica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. *Br. J. Nutr.,* **97**(6): 1187-1195.