**RESEARCH MANAGEMENT CENTRE**
**INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA**

# CHECKLIST FOR COMPLETION OF RESEARCH PROJECT

| NO. | ITEMS | ✓ |
|---|---|---|
| 1. | End of Project Report Form | ✓ |
| 2. | Evidence of Research Report<br>- Printed version of uploaded document in IREP | ✓ |
| 3. | A copy of seminar paper, conference, proceedings, publications | ✓ |
| 4. | Attach Original Receipts<br>*(Kindly sort the receipts according to votes / budgets and properly pasted on separate sheets)* | Has been submitted during advancement report |

*Note:*

*The research project is considered completed once all the above have been submitted and all disbursed funds have been fully reconciled.*

Version: 2.0
Revision: 00
Effective Date: 1/1/2013

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# END OF PROJECT REPORT FORM

## I. RESEARCH DETAILS

TITLE OF RESEARCH: Multivariate calibration of antioxidant activity of *M. charantia* fruits and its fourier transform infrared spectroscopy based fingerprinting

TYPE OF GRANT: RIGS

PROJECT ID: RIGS16-290-0454

PROJECT START DATE: 20-12-2016

PROJECT END DATE:19-12-2017

## II. RESEARCHER DETAILS

PRINCIPAL RESEARCHER: Assoc. Prof. Dr. Alfi Khatib

DEPARTMENT/KULLIYYAH/CENTRE: Department of Pharmaceutical Chemistry/ Kulliyyah of Pharmacy IIUM

PROJECT MEMBERS: Assoc. Prof. Dr. M. Taher bin Bakhtiar

DEPARTMENT/KULLIYYAH/CENTRE: Department of Pharmaceutical Technology/ Kulliyyah of Pharmacy IIUM

## III. RESEARCH ALLOCATION

| Vote | Total Approved budget (RM) | Supplementary Budget Approved (*if any*) (RM) | Total Cumulative Expenditure (RM) | Balance (RM) |
|---|---|---|---|---|
| **V11000** | **0** | | **0.00** | **0.00** |
| **V31000** Travelling Expenses And Subsistence | **3,000** | **-** | **2,723.80** | **276.20** |
| **V27000** Research Materials & Supplies | **16,300** | **-** | **13,530.34** | **2,769.66** |
| **V29000** Professional Services & Other Services including Printing & Hospitality, Honorarium for subjects | **700** | **-** | **3,676.40** | **-2,976.40** |
| | | | | |
| **TOTAL** | **20,000** | **-** | **19,930.54** | **69.46** |

## IV. EQUIPMENT/ASSET PURCHASED

| No. | Item | Placement (*please state specific location)* |
|---|---|---|
| | **None** | |

(*Machinery, books, software, IT equipments e.g. laptop, desktop, printer, scanner, digital camera, and others)*

## V. PROJECT ACHIEVEMENT

1. **Publications** *(International, national, books, chapter in a book, citation, articles, seminar paper, proceedings, etc.*) ***A thesis is considered as a publication***

   **(Format:** *Authors, year, title, full name of journal/conference/proceedings/volume, number of pages***)**

1. Ismail, S. N., Maulidiani, M., Akhtar, M. T., Abas, F., Ismail, I. S., Khatib, A., Mohamad Ali, N. A., Shaari, K. 2017. Discriminative Analysis of Different Grades of Gaharu (*Aquilaria malaccensis* Lamk.) via $^{1}$H-NMR-Based Metabolomics Using PLS-DA and Random Forests Classification Models. Molecules 22, 1612.

2. Lee, S. Y., Mediani, A., Khatib, A., Ismail, I. S., Zawawi, N., Abas, F. 2017. Comparison of partial least squares and random forests for evaluating relationship between phenolics and bioactivities of Neptunia oleracea. Journal of the Science of Food and Agriculture. DOI: 10.1002/jsfa.8462.

3. Khatib, A., Perumal, V., Ahmed, Q. U., Uzir, B. F., Abas, F., Murugesu, S. 2017. Characterization of antioxidant activity of *Momordica charantia* fruit by infrared based fingerprinting. Analytical Letters. doi.org/10.1080/00032719.2016.1261877 (2017).

4. Khatib, A., Perumal, V., Ahmed, Q. U., Uzir, B. F., Murugesu, S. 2017. Low inhibition on alpha glucosidase and xanthine oxidase activities of ethanol extract of *Momordica charantia* fruit. Journal of Pharmaceutical Negative Results 8, 20 (2017).

5. Lawal, U., Leong, S. W., Shaari, K., Ismail, I. S., Khatib, A., Abas, F. 2017. α-Glucosidase Inhibitory and Antioxidant Activities of Different Ipomoea aquatica Cultivars and LC–MS/MS Profiling of the Active Cultivar. Journal of Food Biochemistry 41, Article number e12303.

6. Maulidiani, Rudiyanto, Mediani, A., Khatib, A., Ismail, A., Hamid, M., Lajis, N.H., Shaari, K., Abas, F. Application of BATMAN and BAYESIL for quantitative $^{1}$H-NMR based metabolomics of urine: discriminant analysis of lean, obese, and obese-diabetic rats. Metabolomics 13, 131 (2017).

7. Pariyani, R., Ismail, I. S., Azama, A., Khatib, A., Abas, F., Shaari, K., Hamza, H. 2017. Urinary metabolic profiling of cisplatin nephrotoxicity

andnephroprotective effects of *Orthosiphon stamineus* leaves elucidated by $^{1}$H NMR spectrsocopy. Journal of Pharmaceutical and Biomedical Analysis 135, 20-30.

8. Mediani, A., Abas, F., Maulidiani, M., Khatib, A., Tan, C. P., Ismail, I. S., Shaari, K., Ismail, A. 2017. Characterization of Metabolite Profile in *Phyllanthus niruri* and Correlation with Bioactivity Elucidated by Nuclear Magnetic Resonance Based Metabolomics. Molecules 22 (6), 902.

9. Azam, A. A., Pariyani, R., Ismail, I. S., Ismail, A., Khatib, A., Abas, F., Shaari, H. 2017. Urinary metabolomics study on the protective role of *Orthosiphon stamineus* in Streptozotocin induced diabetes mellitus in rats via $^{1}$H NMR spectroscopy. BMC Complementary and Alternative Medicine **17**, 278.

10. Jambocus, N. G. S., Ismail, A., Khatib, A., Mahomoodally, F., Saari, N., Mumtaz, M. W., Hamid, A. A. 2017. *Morinda citrifolia* L. leaf extract prevent weight gain in Sprague-Dawley rats fed a high fat diet. Food and Nutrition Research 61, 1338919.

11. Alfi Khatib. Application of metabolomics in herbal standardization and bioactivity assessment. In: 2$^{nd}$ International Seminar and Expo on Jamu, 26-27$^{th}$ September 2017, Padjadjaran University, Bandung, Indonesia (Invited Speaker).

- ***Please submit a copy of the publication***

**2. Intellectual Property Rights** *(Patent, Industrial Design, Trademark, Copyright, etc.***)**

None

**3. Human Capital Development** *(PhD, Masters, Research staff with specialty, etc.***)**

1 PhD student (Vikneshwari Perumal)

**4. Commercialization** *(Licensing royalty, spin-off, direct sale, etc.***)**

None

Signature of Principal Researcher: ……

Name : Assoc. Prof. Dr. Alfi Khatib

Date : 26$^{th}$ October 2017

Version: 2.0
Revision: 00
Effective Date: 1/1/2013

## VI. RESEARCH MANAGEMENT CENTRE

**COMMENT:**

_____

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**VERIFICATION ON RESEARCH OUTPUT:**

1. Book ☐
2. Journal ☐
3. Prototype ☐
4. Patent ☐
5. Commercialization ☐
6. Other (Please specify) ☐ _____

Signature: _____

Name: _____

Date: _____

Version: 2.0
Revision: 00
Effective Date: 1/1/2013

# FORMAT OF FULL VERSION OF RESEARCH REPORT TO BE UPLOADED IN THE IREP

*Guidelines for writing the Research Report*

- *Report should be written in 'Times New Roman 12' Font, with 1.5 line spacing*
- *Report should be between 5- 10 pages (excluding references)*
- *Report must be in English (Applicable for Research in Arabic as well)*
- *Any graphic must be in JPEG*

➢ The arrangement of the of Research Report is as follow:

**Project ID/Title:** RIGS16-290-0454/ MULTIVARIATE CALIBRATION OF ANTIOXIDANT ACTIVITY OF MOMORDICA CHARANTIA FRUITS AND ITS FOURIER TRANSFORM INFRARED SPECTROSCOPY BASED FINGERPRINTING

**Project Sponsor:** RIGS IIUM

**Author Name(s):** Assoc. Prof. Dr. Alfi Khatib

**Department/Kulliyyah/Institute/Centre:**

Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia

**Abstract:**

*Momordica charantia* is widely consumed edible fruit. The food and pharmaceutical industries use it as a natural antioxidant. However, the quality control of *M. charantia*-based medicinal products is questionable due to the complexity of metabolites in this fruit. Hence, this study has developed a statistical model in predicting the antioxidant value through the 2, 2-diphenyl-1 picrylhydrazyl radical scavenging activity and ferric reducing antioxidant power based on infrared spectroscopy with attenuated total reflectance. This technique was reliably used for quality control. Six ethanol extracts (0, 20, 40, 60, 80, and 100% in water) of this plant's fruit were prepared. The radical scavenging and ferric reducing antioxidant power activities were measured and the chemical profiling of the extracts was fingerprinted by infrared spectroscopy between 4,000 and 600 cm−1 at a resolution of 4 cm−1. Statistical analysis was developed by correlating the bioactivity and infrared spectra of each extract using orthogonal partial least square discriminant analysis. The C–N, C=O, C–O, C–H, and

OH infrared signals were positively correlated with biological activity. The antioxidant activity of the fruit of *M. charantia* may be due to the presence of several antioxidants that work synergistically.

**Key words:** Antioxidant activity; attenuated total reflectance; fingerprinting; infrared spectroscopy; *Momordica charantia*

## Introduction

### Background:

*Momordica charantia* belongs to the family Cucurbitaceae. It is also known as bitter gourd or bitter melon. *M. charantia* fruit is widely distributed in Asia, Africa, and Caribbean. It is well known for its bitterness and extensively used as backyard fruit. All parts of the plant are bitter. The fruit is oblong shaped and looks like a cucumber. Bitter melon is subdivided into Rita type and Pinakbet type. The Rita type is long, dark green, and less warty while the Pinakbet type is short, warty, and more bitter in taste (Basch et al. 2003; Krawinkel and Gudrun 2006). Our ancestor discovered that bitter melon helps to prevent or control many diseases. Many scientific studies have concluded that M. charantia extracts may be used to treat diabetes, dyslipidemia, and microbial infections. It has also been reported as a potential antioxidant and cytotoxic agent for various types of cancers (Grover and Yadav 2004). Antioxidants play an important role including defending against oxidative damage in the major signaling pathways of cells in the biological system. They are effective free radical scavengers that significantly reduce the concentration of free radicals (Jia et al. 2012). There are several commercially available synthetic antioxidants that include butylated hydroxyanisole, butylated hydroxytoluene, and tertbutyl hydroquinone; however, the use of these synthetic antioxidants is restricted due to toxic side effects. Moreover, it has also been shown that they promote the development of cancer in rats (Meenakshi et al. 2011). This finding has greatly reinforced efforts for the development of alternative antioxidants from the natural origins such as medicinal herbs (Meenakshi et al. 2011). The use of medicinal herbs has been facing the problem of quality control because plants contain mixtures of metabolites that may simultaneously work synergistically or antagonistically. Furthermore, the quality of herbs cannot be solely relied on a few metabolites. Thus, the best approach to control the quality is by determining all metabolites in the sample. A particular method which is based on the analysis of individual compounds has high cost because many

measurements may be required for multiple metabolites. Hence, a simple and rapid analytical method should be developed to analyze these materials. One approach to characterize herbs is the use of infrared spectroscopy with attenuated total reflectance. This approach characterizes functional groups of metabolites in the samples. Infrared with attenuated total reflectance has been used frequently in fingerprinting (Aharoni et al. 2002). Compared to nuclear magnetic resonance, infrared spectroscopy is inexpensive, nondestructive, and requires a small amount of sample (Roggo et al. 2007). The results from this approach are measured based on the vibration frequencies of the molecular bonds (Smith 1999; Roggo et al. 2007). Chemometric analysis by infrared with attenuated total reflectance is rapid for a variety of herbal products (Cozzolino et al. 2009). Lam et al. (2009) reported this approach to determine the capacity of antioxidants in blueberry, red grape, and blackberry wine. The antioxidant properties of the fruit were correlated to the presence of hydroxyl groups, phenolic rings, and sugars. Similar work was for seaweed (Meenakshi et al. 2011), Pereskia bleo (Sharif et al. 2014), and Sambuci flos (Clara et al. 2016).

**Objective:**

The objevtive of this study was to develop a validated regression model to characterize the antioxidant activity of *M. charantia* using infrared spectroscopy with attenuated total reflectance.

**Methodology:**

*Chemicals*

Ethanol, hydrochloric acid, acetic acid, and acetone were purchased from R & M Marketing (Essex, UK). Ascorbic acid, 2,2-diphenyl-1 picrylhydrazyl, 2,4,6-tris (2-pyridyl)-s-triazine, iron (III) chloride hexahydrate, and sodium acetate were obtained from Sigma-Aldrich Chemie (Missouri, USA).

*Samples and preparation*

The 12-week-old fruit of *M. charantia* (pinakbet type) was randomly collected from a farm in Perak, Malaysia. A sample was deposited in the Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia on 8 June 2014 for species verification. The fruit was cut into small pieces to remove seeds. The fruit was washed and lyophilized after

treatment with liquid nitrogen. The dried fruit was pulverized into a fine powder and stored at −80°C until analysis. A total of 36 samples for extraction was arranged in which 6 duplicates at 6 ethanol concentrations were prepared. The samples were placed inside a conical flask with a mass of 5 g each. The samples mixed with 150 mL of 0, 20, 40, 60, 80, or 100% ethanol in water and sonicated for 30 min. The samples were passed through filter paper (No. 1, Whatman International, Maidstone, UK) followed by the removal of solvent using a rotary evaporator (Buchi, Flawil, Switzerland) at 40 °C before freeze-drying. The samples were stored at −80°C until analysis (Javadi et al. 2014).

*Radical scavenging*

The radical scavenging activities of six concentrations of *M. charantia* were measured according to the method of Karthivashan et al. (2013) with minor changes. A sample of 20 µL was added to 80 µL of a radical scavenging solution (i.e., 2,2-diphenyl-1 picrylhydrazyl, 2,4,6-tris (2-pyridyl)-s-triazine) (0.2 mmol in water). This plate was kept for 10 min in darkness at room temperature. The absorbance of the mixture was measured at 540 nm using a microplate reader (Tecan, Männedorf, Switzerland). As for the blank, 20 µL of water was added to 80 µL of the radical scavenging solution (0.2 mmol in ethanol). Ascorbic acid was used as standard positive control. The half maximal inhibitory concentration value of the radical scavenging activity was determined as a concentration of the sample at which 50% radical scavenging activity was detected. It was calculated based on the regression line by plotting radical scavenging activity (%) versus various concentrations of the sample. The measurements were performed using six replicates and the mean and standard error were reported.

*Ferric reducing antioxidant power assay*

The total antioxidant capacity of the ethanol extracts of the fruit of *M. charantia* was determined by the ferric reducing antioxidant power method adapted from Szydlowska-Czerniak et al. (2011) with some modifications. Briefly, the ferric reducing antioxidant power reagent consisting of 2.5 mL of 10 mM 2,4,6-tris (2-pyridyl)-s-triazine solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3, and 25 mL of pH 3.6 0.1 M acetate buffer was prepared freshly and was incubated for 10 min at 37°C. A sample of 20 µL and 40 µL of ferric reducing antioxidant power reagent were added to 140 µL of distilled water in a 96-well plate. A blue color was observed and maintained in darkness at room temperature for 20

min. The absorbance of the mixture was measured at 593 nm against the reagent blank (40 µL of ferric reducing antioxidant power reagents made up to 200 µL distilled water) using a microplate reader (Tecan, Männedorf, Switzerland). A calibration curve was prepared using ascorbic acid. The results were reported as total antioxidant capacity in ascorbic acid equivalents by interpolation of the net absorbance. The results were corrected for dilution and expressed as ascorbic acid equivalent microgram of ascorbic acid per gram.

*Infrared spectroscopy with attenuated total reflectance*

A Fourier transform infrared spectrometer (PerkinElmer Inc., Massachusetts, USA) equipped with a horizontal attenuated total reflectance device with a diamond crystal was used for analysis. The instrument was allowed to equilibrate at room temperature (22°C) before measurements. A small mass of each sample was placed neatly on the diamond crystal with a clean spatula. The infrared spectra were analyzed between 600 and 4,000 cm−1 at a resolution of 4 cm−1. The data were processed using Perkin Elmer Spectrum version 10.03.09 software (Massachusetts, USA). The spectra were collected using a rapid scan for each sample (Sharif et al. 2014).

Statistical analysis Spectra were converted into ASCII format before baseline correction was performed and pooled with ferric reducing antioxidant power and radical scavenging activity. The data were subsequently transferred into Microsoft Excel format and imported into Simca P þ14.0 (Umetrics, Umeå, Sweeden) for multivariate data analysis. Orthogonal partial least square discriminant analysis was used in accordance with antioxidant activity and infrared spectra. The data were treated prior to analysis using first, second, and third filter derivatives. It was ultraviolet-scaled and centered to be processed using orthogonal partial least square discriminant analysis. Significant differences were evaluated by one-way ANOVA with Tukey comparison at 95% confidence interval using Minitab 14 (Minitab Inc., State College, PA, USA).

*Antioxidant activity of M. charantia fruit*

*M. charantia*'s fruit of 100 g was collected from local markets. The fruit was washed, cleaned carefully prior removing the seeds, and an 80% ethanol extract was prepared with six replicates for each sample. Each extract was analyzed for 2,2-diphenyl-1 picrylhydrazyl, 2,4,6-tris (2-pyridyl)-s-triazine antioxidant scavenging activity, and ferric reducing

antioxidant power assay activity. Infrared spectroscopy with attenuated total reflectance was used to characterize the antioxidant activity using multivariate analysis.

**Findings:**

The past few decades have seen the rapid development of evaluation of herbal activity using fingerprinting (World Health Organization 1998). Examples of commonly used techniques The past few decades have seen the rapid development of evaluation of herbal activity using fingerprinting (World Health Organization 1998). Examples of commonly used techniques include gas chromatography–mass spectroscopy (GC-MS), nuclear magnetic resonance (NMR), and high-performance liquid chromatography–mass spectroscopy (HPLC-MS). However, these instruments are expensive, require high maintenance, and are limited in developing countries (Cozzolino et al. 2009). Thus, infrared spectroscopy with attenuated total reflectance is considered to be more suitable for the analysis of medicinal herbs. It is inexpensive, commonly available, and uses vibration energy to identify significant functional groups responsible for biological activity (Roggo et al. 2007). Infrared spectroscopy with attenuated total reflectance spectroscopy is widely used in industrial quality control. Nurrulhidayah et al. (2013) reported chemometrics and infrared spectroscopy to detect adulteration in food. Infrared spectroscopy with attenuated total reflectance provided specific fingerprinting of dairy products that were suitable for quality control. Yang et al. (2005) reported the use of infrared spectroscopy-based fingerprinting for quality control to distinguish butter and other fats.

*Extraction yield*

The yield of extractions is listed in Table 1. It shows that 80% ethanol extract of *M. charantia* resulted in higher yield of extraction . Mixtures of alcohol and water are more efficient in facilitating higher yield of extraction compared to monocomponent solvents (Spigno et al. 2007). The trend of yield of extraction was 80 >0 >20 >60 >100 >40%. It is noteworthy that the trend of the yield of extraction was not consistent with the trend of ferric reducing antioxidant power and radical scavenging antioxidant activities. The bioactivity of the sample was not directly proportional to the yield of extraction because it depends on the profile of the metabolites present in the sample.

*Radical scavenging*

The scavenging activity was obtained in a concentration-dependent manner. The ability of various concentrations of ethanol extract of the fruit of *M. charantia* to scavenge free radicals is demonstrated in Table 1. The 80% ethanol extract showed the strongest inhibition in the half maximal inhibitory concentration of radical scavenging activity value of 0.37 mg/mL ($p < 0.00$) compared to standard ascorbic acid at 0.02 mg/mL. This result was followed by the 100, 60, 40%, and the lowest scavenging activity was found in 20 and 0% ethanol extracts of *M. charantia* fruit with the values of 0.53, 1.05, 1.09, 1.10, and 1.10 mg/mL, respectively. The differences in the half maximal inhibitory values for the concentrations of ethanol extract may be due to differences in type and number of metabolites present in the extract due to variation in polarity of solvent in the extraction (Javadi et al. 2014).

**Table 1.** Comparison of the extraction yield, ferric reducing antioxidant power, and radical scavenging activity of *Momordica charantia* fruit ($n = 6$).

| Extract (%) | Ferric reducing antioxidant power, equivalent microgram of ascorbic acid per gram | Radical scavenging activity, half maximal inhibitory concentration (mg/mL) | Extraction yield, % |
|---|---|---|---|
| 0 | $54.27 \pm 0.60^{a}$ | $1.10 \pm 0.08^{a}$ | $40.36 \pm 3.23^{a}$ |
| 20 | $65.32 \pm 0.23^{b}$ | $1.10 \pm 0.04^{a}$ | $38.51 \pm 1.10^{b}$ |
| 40 | $85.51 \pm 1.04^{c}$ | $1.09 \pm 0.08^{a}$ | $23.30 \pm 1.26^{b}$ |
| 60 | $86.11 \pm 2.16^{c}$ | $1.05 \pm 0.10^{ab}$ | $35.32 \pm 2.30^{bc}$ |
| 80 | $113.85 \pm 0.94^{d}$ | $0.37 \pm 0.07^{d}$ | $62.03 \pm 1.27^{cd}$ |
| 100 | $112.31 \pm 1.34^{e}$ | $0.53 \pm 0.06^{cd}$ | $24.71 \pm 3.14^{d}$ |
| Ascorbic acid | $114.58 \pm 1.73^{d}$ | $0.02 \pm 0.01^{e}$ | Not determined |

Values in each column with different subscript letters are significantly different ($p < 0.00$).

Ferric reducing antioxidant power activity

In ferric reducing antioxidant power assay, compounds that are rich in antioxidants exert their action by breaking the free radical chain by donating a hydrogen atom (Qader et al. 2011). The results are expressed quantitatively in term of ascorbic acid equivalents microgram of ascorbic acid per gram. In the current study, ascorbic acid was used as standard. Table 1 shows that the 80% ethanol extract of the fruit of *M. charantia* showed the highest antioxidant capacity with 113.85 ascorbic acid equivalent microgram of ascorbic acid per gram ($p < 0.00$) and was higher than the antioxidant activity of ascorbic acid (114.58 ascorbic acid equivalent microgram of ascorbic acid per gram). This value was followed by 100, 60, 40, 20 and 0% ethanol extracts of the fruit of *M. charantia* with the values of 112.31, 86.11, 85.51, 65.32, and 54.27 ascorbic acid equivalents microgram of ascorbic acid

per gram, respectively. These results show that *M. charantia* fruit exhibited antioxidant activity because of their capacity to scavenge various free radicals and reduce ferric. This result was consistent with Qader et al. (2011) in which the water extract presented the weakest antioxidant potential in contrast to ethanol extract of the fruit of *M. charantia*. Incongruent to our results, similar findings were also obtained by Horax et al. (2010) who stated that 80% ethanol extracts of the pericarp and seeds of *M. charantia* exhibited the highest antioxidant activity compared to other extracts. This result may be because most medicinal herbs contain phenolic compounds at high concentrations that contribute to the antioxidant activity. The total phenolics was found to increase with the ethanol concentration from 0 to 80%. Moreover, the same total phenolic content was found to decrease when the ethanol concentration was increased by 95%. This result may be due to variations in solvent polarity in the study that may also be responsible for extracting other phenolics during extraction from the pericarp and seeds of *M. charantia* (Marinova and Yanishlieva 1997).

Infrared spectroscopy

The entire infrared spectrum was used in this study to provide more accurate interpretation. Promising results were obtained using the entire infrared region to develop a predictive orthogonal partial least square—discriminant analysis model for the antioxidant activity of the fruit of *M. charantia*. Representative infrared spectra are shown in Figure 1. The base groups, vibrational modes, and attribution of each assigned peak are tabulated in Table 2. The functional groups were confirmed with the results of Pavia et al. (2014). The peaks from 3,220 to 3,540 cm−1 were assigned to O-H bonds as alcohols, phenols, and carboxylic acid groups. The value at 2,925 cm−1 was due to C-H stretching of alkanes, alkenes, aromatics, and aldehydes. The region from 1,720 to 1,820 cm−1 was due to carbonyl groups as aldehydes, ketones, carboxylic acids, and esters. Absorption from 1,550 to 1,640 and 1,350 cm−1 corresponds to nitro groups. The S=O of sulfones, sulfonyl chlorides, sulfates, and sulfonamides were between 1,300 and 1,375 cm−1. The peaks from 770 to 920 cm−1 represent the C-H out of plane vibrations from alkanes and aromatics (Pavia et al. 2014)

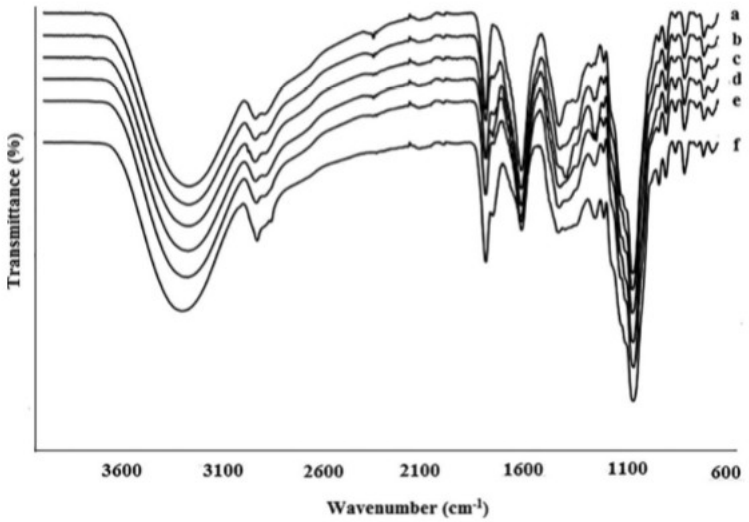


**Figure 1.** Representative infrared spectra of *Momordica charantia* fruit extracts using (a) 0%, (b) 20%, (c) 40%, (d) 60%, (e) 80%, and (f) 100% ethanol in water.

Multivariate analysis

Multivariate analysis was used to evaluate the differences between various concentrations of *M. charantia* fruit ethanol extract as visual inspection of the infrared spectra was insufficient to support the findings. The profile of each extract was correlated to its antioxidant activity to establish a validated regression model. This regression model is crucial in quality control and usually used for predictive purposes. Good accuracy depends upon the validation of model (Mourad, Bertrand-Krajewski, and Chebbo 2005). The orthogonal partial least square is considered the most suitable when dealing with large data sets with multiple variables. Orthogonal partial least squares are extensively used in biochemistry and biology (Eriksson 2006). The orthogonal partial least square model improves the detection of outliers in the score to avoid disturbance in the calculation. This approach also reduces the time of internal iteration by rapid calculations and decreases the total number of components by removing orthogonal principal components (Sadeghi-Bazargani, Banani, and Mohammadi 2010).

**Table 2.** Infrared spectral assignments of *Momordica charantia* ethanol extracts based on Pavia et al. (2014).

| Wavenumber ($cm^{-1}$) | Functional group | Vibrational mode | Responsible compounds |
|---|---|---|---|
| 3,220–3,540 | O–H | Stretching | Alcohol, phenol or carboxylic acid |
| 2,925 | C–H | Stretching | Alkanes or alkenes or aromatics or aldehyde |
| 1,720–1,820 | C=O | Stretching | Ester, carboxylic acid, ketone, aldehyde |
| 1,550–1,640 and 1,350 | N=O | | Nitro |
| 1,300–1,375 | S=O | | Sulfones, sulfonyl chlorides, sulfates sulfonamides |
| 1,000–1,210 | C–O | | Alcohols, ethers, esters, carboxylic acids, anhydrides |
| 700–920 | C–H | Out of plane | Alkenes and aromatics |

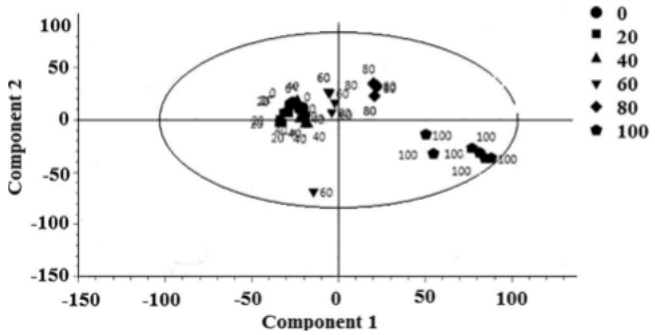


**Figure 2.** Differentiation of *Momordica charantia* fruit extracted with 0, 20, 40, 60, 80, and 100% aqueous ethanol based on orthogonal partial least square discriminant analysis. The fraction of the sum of square of all *X* values explained by principal component 1 is 0.467. The fraction of the sum of the square of all *X* values explained by orthogonal component 1 is 0.304.

Supervised orthogonal partial least squares—discriminant analysis is a variation of partial least square discriminant analysis (PLS-DA) that helps to delete the unwanted portions of X variables that are not correlated with Y variables (Trygg and Wold 2002). Orthogonal partial least squares—discriminant analysis helps to develop the correlation between two matrices. In this study, the first matrix is the chemical information from the infrared spectra (X). The second matrix is the antioxidant activity as the ferric reducing antioxidant power and radical scavenging activity (Y). The multivariate data analysis shows the classification of various concentrations of *M. charantia* and the relationship between observed X and the predicted Y in Figure 2. Twenty-four observations and variables (X ¼3600, Y ¼2) were developed. The most active sample is in the positive quadrant that is 80 and 100% of ethanol extracts of *M. charantia* fruit. Meanwhile, the negative quadrant of the plot revealed that 0, 20, 40, and 60% of ethanol extracts of the fruit of *M. charantia* have low antioxidant activity. The line loading plot in Figure 3 explains the correlation of potential wavenumber from the infrared spectra (X variable) to the antioxidant activity (Y variable). The plot evaluates the spectral features that increase or reduce the antioxidant activity. The peaks with negative first predictive loading values correlate to the antioxidant activity and vice versa. Thus, the peaks from 3,220 to 3,540, 1,720 to 1,820, 1,000 to 1,210, and 770 to 920 cm−1 corresponding to the signals from the antioxidant compounds. These peaks were assigned to the O–H, C=O, C–O, and C–H (out of plane), respectively, and attributed to alcohols, sugars, alkaloids, triterpenoids, saponins, and phenolic.

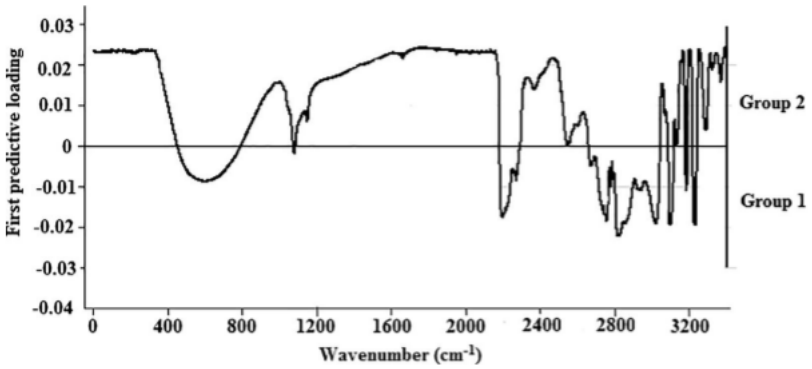


**Figure 3.** Line loading plots of the orthogonal partial least square model based on orthogonal partial least square discriminant analysis.

Antioxidant metabolites

The phenolic compounds that have been isolated from *M. charantia* include tannic acid, p-coumaric, gentisic acid, gallic acid, epicatechin, chlorogenic acid, caffeic acid, benzoic acid, and (þ)-catechin (Haque et al. 2011). Kubola and Siriamornpun (2008) reported that gallic acid is found in all parts of *M. charantia*. Furthermore, a wide range of plants contains phenolics. Rice-Evans et al. (1995) stated that phenolic compounds may be antioxidants by acting as reducing agents, singlet oxygen quenchers, and have redox properties and metal chelation potential. Phenolics have at least one hydroxyl group attached to an aromatic ring (Okigbo et al. 2009). Various studies have been reported on antidiabetic, antimutagen, antitumor, anticarcinogenic, and anti-inflammatory activities of phenolics from *M. charantia* (Budrat and Shotipruk 2009; Paul and Raychaudhuri 2010). The antioxidant activity of catechin and catechin gallate esters of green tea was shown to involve the linkage of gallic acid to the epicatechin or epigallocatechin through esterification at the third position (Salah et al. 1995). Aglycones and glycones are flavonoids ubiquitously found in plants. Several phenolic hydroxyl groups are attached to flavonoid rings, and in glycosylation process, a carbohydrate is attached to a hydroxyl or other functional group of flavonoid. The glycosylation process enables flavonoids to be more reactive toward free radicals. Glycosylation commonly occurs at the 7-hydroxyl group in flavones, isoflavones, and dihydroxyflavone; 3- and 7-hydroxyls in flavonols and dihydro flavonols; and 3- and 5-hydroxyls in anthocyanidins (Rice-Evans et al. 1995). Further modification in biosynthesis during glycosylation enables the production of C-glycosides in which a sugar is directly bonded to the aromatic carbons of the flavonoid which increases the antioxidant activity of flavonoid glycosides. Shan et al. (2012) reported that flavonoids from the ethanol extract of

*M. charantia* through modified supercritical carbon dioxide extraction displayed higher antioxidant activity than flavonoids obtained through conventional solvent extraction. The radical scavenging activity of *M. charantia* L. fruit obtained by supercritical carbon dioxide extraction or conventional solvent extraction increased with the flavonoid concentration. Jain et al. (2008) reported that M. dioica Roxb. leaves have a hepatoprotective action against carbon tetrachloride-induced hepatic damage in rats. The hepatoprotective activity was attributed to the presence of flavonoids in the leaf extracts that enhance the antioxidant activity. One sugar that possesses antioxidant activity is trehalose, a disaccharide composed of two molecules of glucose joined by glycosidic bond (Aoki et al. 2010). Trehalose has a suppressive effect on subarachnoid hemorrhage, enhances the clearance of A53T mutant alpha-synuclein in PC12 cells mediated through the macroautophagy pathway, possesses anti-inflammatory properties, and has also been reported to be beneficial in Parkinson patients (Chun-Lin et al. 2013). A recent study reported that trehalose from *M. charantia* fruit prevents the progression of diabetes (Arai et al. 2010). Recently, Kuo and Chien (2015) reported that guava juice mixed with trehalose showed less oxidative damage in the kidneys and pancreas of diabetic female Wistar rats after 4 weeks. Chaturvedi (2012) showed that *M. charantia* repaired the pancreatic b-cells due to its strong antioxidant activity. Trehalose has also been reported to exhibit antioxidant activity through a reduction of oxidation of unsaturated fatty acids (Oku et al. 2005). The isolated triterpenoids from the stems and fruits of *M. charantia* have been reported to exhibit strong antioxidant activity based on the determination of free radical scavenging activity, 2, 20-azo-bis (3-ethylbenzothiazoline-6-sulphonic acid), xanthine oxidase inhibitory activity, superoxide anion scavenging capacity, and oxygen radical absorbance capacity. The isolated compounds were 7b-O-[b-D-glucopyranosyl(1-6)-b-D- glucopyranosyl]-3b-acetyl-cucurbita-5,22,24-trien-19b-al (cucurbitane-type triterpene glycoside) and 23,24,25,26,27-pentanorcucurbita-5-en-3,7-dioxo-22-oic acid. The antioxidant capacity of the former was more likely due to glycosidation with two moles of b-D-glucose. Chiung et al. (2010) isolated a new multiflorane triterpenoid and two new cucurbitane triterpenoids from the stems of *M. charantia* that were reported to show antioxidant activity. These new compounds were shown to be 3b-hydroxymultiflora-8-en-17-oic acid, cucurbita-1(10),5,22,24-tetraen-3α-ol, and 5b,19b-epoxycucurbita-6,22,24-trien- 3α-ol. However, the latter compound exhibited weak antioxidant activity because of the presence of an epoxy group between C-5 and C-19 that was responsible for weakening the activity of xanthine oxidase. Charantin, momordenol,

and momordicilin are examples of triterpenoids present in *M. charantia* fruit (Hazarika et al. 2012). Numerous studies have been reported on the antidiabetic, antimicrobial, and antilipidemic activities of these compounds. However, there is no study reported on the antioxidant potential of these compounds. Saponins are well-known bioactive phytochemicals those have been thoroughly investigated for pharmacological activities, including antimicrobial, cytotoxic, anti-inflammatory, and immunostimulatory (Keller et al. 2011). However, antioxidative studies of saponins isolated from *M. charantia* are limited. The well-known saponins momordin and momorcharside are present in *M. charantia* fruit. The antioxidant potential of M. balsamina was due to the presence of these saponins through radical scavenging activity, lipid peroxidation with thiobarbituric acid, and reducing power assays (Faujdar et al. 2013). Tan et al. (2014) reported a strong correlation (fraction of total sums square ¼0.90) between saponins from three optimized powdered extracts (aqueous spray dried, aqueous freeze-dried and ethanol freeze-dried) of *M. charantia* with the total antioxidant assay measured by 2, 2-diphenyl-1 picrylhydrazyl radical scavenging activity, 2,20-azo-bis (3-ethylbenzothiazoline-6-sulfonic acid), and ferric reducing power. The mechanism of saponins was due to the indirect scavenging on free radicals and metal ion chelation (Houng et al. 1998). The metabolites that contain C–N functionalities from 1,236 to 1,296 cm−1 are alkaloids. Blycosidic alkaloid vicine has been reported to be in the seeds of *M. charantia* (Nagarani et al. 2014). The mechanism of vicine as an antioxidant agent may be through interfering with lipids or other oxidations by rapid donation of hydrogen atoms to radicals because this compound may serve as a chain breaker and free radical acceptor (Perron and Brumaghim 2009). Furthermore, El-Maksoud et al. (2013) showed that the ability to inhibit peroxidation and reaction with peroxyl radicals increases with O-deglycosylation of vicine. However, this compound has also been reported to cause glucose-6-phosphate dehydrogenase deficiency (Basch et al. 2003). Hence, a further study is required to ensure the safe consumption of *M. charantia* for patients with favism.

Validation of the model

The reliability of a predictive model depends upon the validation of the calibration model. It is important to avoid overfitting to obtain reliable results (Trivedi and Iles 2012). Model validation may be performed using cross-validation (Trivedi and Iles 2012) that helps to estimate the overall predictive power of the model and evaluate the significance of the latent

variable (Eriksson 2006). Chemometrics requires cross-validation to create a permuted response that is then compared to the real response (Trivedi and Iles 2012). Figure 4a illustrates the permutation of 0, 20, 40, and 60% of ethanol extracts of *M. charantia* fruit and Figure 4b shows the results for 80 and 100% of ethanol extracts of the sample. The model was validated by comparing the predictive ability of the model intercept values when the original Y-intercept values were randomly assigned to the individuals (Garcia-Perez et al. 2010). The fraction of the total sum of squares intercept for group 1 was 0.0874 and the predictive ability of the model intercept was −0.372. However, the fraction of the total sum of square intercept for group 2 was 0.0891 and the predictive ability of the model intercept was −0.362. These results demonstrate the validity of model for further analysis. The validity of both models was demonstrated because the intercepts for the fraction of total sum of the square intercept Y-value and the predictive ability of the model intercept Y-value were below 0.3 and −0.05, respectively (Eriksson 2006). The external validation of the calibration model was obtained through the introduction of external samples. This is essential to evaluate the reliability and predictability of the model (Sharif et al. 2014). Table 3 shows the actual and predicted values for ferric reducing power activity and free radical scavenging activity. The 80% ethanol extract provided the highest accuracy for all samples for ferric reducing power and radical scavenging activity.

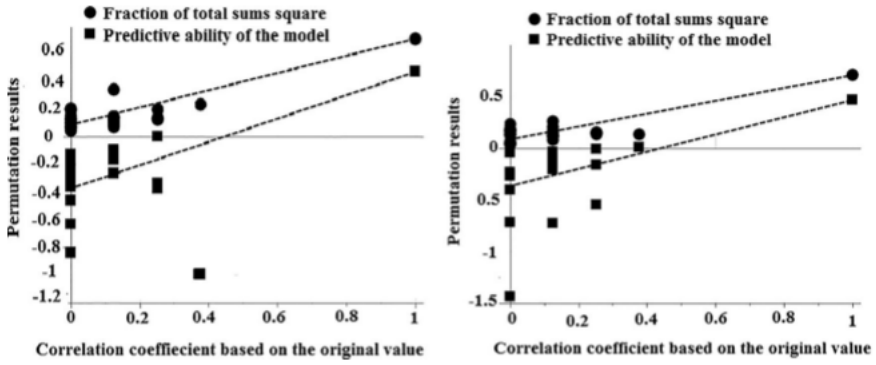


**Figure 4.** (a) Permutation results of samples from group 1 with the 0, 20, 40, and 60% aqueous ethanol extracts of *Momordica charantia* fruit. The intercept of the fraction of the total sum of the squares was 0.0874 while the intercept of the predictive ability of the model was −0.372. (b) Permutation results of samples from group 2 with the 80 and 100% aqueous ethanol extracts of *Momordica charantia* fruit. The intercept of the fraction of total sum of the squares was 0.0891 while the intercept of the predictive ability of the model was −0.362.

**Table 3.** Measured and predicted ferric reducing power assay and radical scavenging activity of 80% ethanol extracts of *Momordica charantia* fruit from Kuantan, Malaysia ($n = 4$).

| Sample | Actual radical scavenging activity, equivalent microgram of ascorbic acid per gram | Predicted radical scavenging activity | Actual ferric reducing power, half maximal inhibitory concentration (mg/mL) | Predicted ferric reducing power |
|---|---|---|---|---|
| 1 | $0.42 \pm 0.05^{a}$ | Highly active | $113.74 \pm 2.07^{ab}$ | Highly active |
| 2 | $0.39 \pm 0.09^{a}$ | Highly active | $109.64 \pm 1.94^{ab}$ | Highly active |
| 3 | $0.42 \pm 0.10^{a}$ | Highly active | $109.67 \pm 0.84^{ab}$ | Highly active |
| 4 | $0.51 \pm 0.05^{a}$ | Highly active | $109.39 \pm 0.26^{ab}$ | Highly active |
| 5 | $0.43 \pm 0.02^{a}$ | Highly active | $115.34 \pm 1.14^{ab}$ | Highly active |
| 6 | $0.54 \pm 0.10^{a}$ | Highly active | $114.17 \pm 1.21^{ab}$ | Highly active |
| 7 | $0.49 \pm 0.04^{a}$ | Highly active | $115.86 \pm 6.46^{b}$ | Highly active |
| 8 | $0.55 \pm 0.04^{a}$ | Highly active | $105.48 \pm 0.69^{a}$ | Highly active |
| 9 | $0.55 \pm 0.04^{a}$ | Highly active | $106.57 \pm 1.42^{ab}$ | Highly active |
| 10 | $0.52 \pm 0.05^{a}$ | Highly active | $110.46 \pm 2.82^{ab}$ | Highly active |

Values in each column with different subscript letters are significantly different ($p$-value for radical scavenging is $p > 0.50$, $p$-value for ferric reducing power is $p > 0.50$).

Hence, this study developed a rapid validated model with good accuracy with potential application for quality control in the herbal industry to provide consistent quality of *M. charantia* fruit extracts.

**Conclusion:**

This study demonstrated the use of infrared spectroscopy with attenuated total reflectance to characterize the antioxidant activity of M. *charantia* fruit. This method is suitable for quality control of *M. charantia* fruit-based herbal preparations. The constructed model identifies the peaks responsible for the activity. The C–N, C=O, C–O, C–H, and OH infrared peaks were positively correlated with antioxidant activity. This biological activity may be due to the presence of several active components that act synergistically.

## Output:

Calibarated statistitical model for antoxidant prediction of *M. charantia* fruit.

## Future Plan of the research:

This model will be used to optimize the processing condition of the fruit.

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## (A) FULL VERSION OF RESEARCH REPORT

**(Compulsory for the principal researcher to prepare this report for the presentation of the result of the research project at the IIUM Seminar on Research Findings)**

- Kindly also submit a full-version of the research report

The above report is the full version of the research report.