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Comparative Phytochemical Profiling and Antioxidant Evaluation of Different Plant Parts of *Momordica charantia* (Cucurbitaceae) from Borneo Island

Norhayati Yusuf[1,3], Nur Fazirah Mohammad Ali[1], Asnuzilawati Asari[1,2], Maulidiani Maulidiani[1,2] and Nurul Huda Abdul Wahab[1,2*]

[1]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[2]*Advanced Nano Materials (ANoMa) Research Group, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

[3]*Biological Security and Sustainability (Bioses) Research Interest Group, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*

Momordica charantia, commonly known as bitter gourd, belongs to the Cucurbitaceae family and is widely distributed across tropical regions worldwide. In Malaysia, it is locally referred to as *peria katak* and is traditionally used as food and medicine. Despite extensive reports on its therapeutic potential, there remains limited comparative data on how different plant parts and solvent systems influence the recovery of bioactive compounds and antioxidant activity, particularly for Malaysian varieties. This gap limits the scientific validation of *M. charantia* as a source of nutraceuticals and pharmaceuticals. In this study, the leaves, fruits, and seeds of *M. charantia* collected from Tawau, Sabah, were extracted using solvents of varying polarity hexane, ethyl acetate, and methanol and evaluated for phytochemical composition and antioxidant potential. Antioxidant properties were assessed through 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, carotenoid content, and α-tocopherol concentration. Among the solvents, methanol yielded the highest extraction percentages (11.05 ± 0.35% from leaves, 8.10 ± 0.16% from fruits, and 4.68 ± 0.057% from seeds). Qualitative phytochemical screening revealed alkaloids, flavonoids, reducing sugars, carbohydrates, steroids, saponins, tannins, phlobatannins, and phenolics in the methanol extract of leaves. Furthermore, methanol leaf extract demonstrated the strongest antioxidant capacity, with 98.43 ± 0.09% DPPH radical scavenging activity, alongside elevated α-tocopherol (4.65 ± 0.01 mg/g.fwt) and carotenoid levels (44.18 ± 1.81 mg/g.fwt). These findings demonstrate that the choice of solvent and plant part significantly affects phytochemical recovery and antioxidant potential. The results provide evidence to address the current gap and support the pharmaceutical exploration of *M. charantia* as a promising natural source of antioxidants.

Keywords: Bitter gourd; phytochemical; secondary metabolites; antioxidants; DPPH

I. INTRODUCTION

Momordica charantia (Figure 1) commonly known as bitter melon, bitter gourd, balsam pear, or African cucumber, is a tendril-climbing annual vine belonging to the order Cucurbitales, family Cucubitaceae and genus Momordica (Desai & Tatke, 2015). This species is widely distributed in tropical regions, including Malaysia, India, China, tropical Africa, Thailand, and America (Raghavan & Anilakumar, 2015). In Malaysia, it is locally referred to as *peria katak*. It is cultivated primarily for its fruit, which is used as a flavouring agent in various Asian dishes. The fruits, flowers, and young shoots are commonly added to soups for their

*Corresponding author's e-mail: nhuda@umt.edu.my

slight bitterness, while Indian cuisine often blanches or soaks the fruit in salt water to reduce bitterness (Saeed *et al.*, 2018). It can also be canned, pickled, dehydrated, or deep-fried. Traditionally, bitter gourd is widely recognised as a folk medicine for diabetes across Asia, South America, India, and East Africa. Besides the fruit, its roots, leaves, and vines treat toothaches, diarrhoea, and skin infections. Bitter gourd tea, known as *gohyah*, made from dried fruit slices, is gaining popularity as an herbal remedy. The plant's medicinal potential continues to attract interest for its therapeutic applications (Jia *et al.*, 2017).

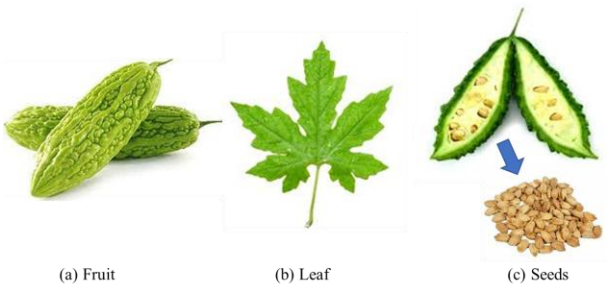

Figure 1. a) Fruit, b) Leaf and c) Seeds of *Momordica charantia*

Research has shown that this plant contains an insulin-like compound, often called "plant insulin," which helps lower blood and urine glucose levels (Janagal *et al.*, 2018). Additionally, it exhibits anti-cholesterol (Saeed *et al.*, 2018), anti-cancer (Bai *et al.*, 2016), anti-dementia (Joshi *et al.*, 2017), anti-bacterial & anti-fungal (Mahmood *et al.*, 2019), antioxidant and anti-inflammatory (Bortolotti *et al.*, 2019) activities. Various parts of the plant, especially fruits and seeds, contain over 60 bioactive compounds effective against more than 30 diseases (Kole *et al.*, 2020).

The bioactive profile of *Momordica charantia* includes primary metabolites such as sugars, proteins, and chlorophyll, and a diverse range of secondary metabolites, including phenolics, carotenoids, cucurbitane type triterpenoids, alkaloids, and saponins (Cortez-Navarrete *et al.*, 2021). These secondary metabolites are primarily responsible for the nutraceutical properties of bitter gourd, which, although not directly contributing to its nutritional value, exert beneficial physiological effects such as antioxidant, antidiabetic, and anticancer activities (Daniel *et al.*, 2014; Chou *et al.*, 2022). Among these, cucurbitane type triterpenoids are recognised as the major marker compounds of *M. charantia*, with charantin, momordicosides, and momordicines being widely reported as characteristic constituents of the species (Tan *et al.*, 2008; Fang *et al.*, 2012; Chou *et al.*, 2022). Charantin, in particular, has been extensively studied for its hypoglycemic activity and is often used as a phytochemical marker for standardisation of bitter gourd extracts (Grover & Yadav, 2004). Additionally, polypeptide-p, an insulin like peptide, has been isolated and reported to exert blood glucose-lowering effects (Joseph & Jini, 2013).

Previous studies have documented the phytochemical composition and antioxidant properties of *M. charantia* across different regions and plant parts. For example, Kubola and Siriamornpun (2008) reported high phenolic and flavonoid contents in methanolic extracts of seeds and pulp, while Sathishsekar and Subramanian (2005) highlighted the antioxidant and hypoglycemic effects of leaf extracts. In China, Fang *et al.* (2012) identified and characterised cucurbitane-type triterpenoids from fruits, whereas Chou *et al.* (2022) examined the antioxidant and anti-inflammatory activities of bitter gourd extract in Taiwan. However, despite these efforts, there remains limited documented information on the phytochemical composition and antioxidant properties of *M. charantia* cultivated in Malaysia, particularly when considering the influence of solvent polarity and plant part selection.

The increasing demand for natural antioxidants in pharmaceutical applications highlights the need for further investigation. One of the major challenges in phytochemical research is the isolation and biological evaluation of secondary metabolites, as some extracted compounds may not exhibit significant biological activity (Jayasinghe *et al.*, 2003). Additionally, using a single solvent system and limited antioxidant assays may not provide a comprehensive understanding of its antioxidant capacity.

This study aims to analyse the phytochemical composition of *M. charantia* cultivated in Malaysia and evaluate its antioxidant potential. Unlike previous studies that focused on extractions using single solvents, this research employs three organic solvents: hexane, ethyl acetate, and methanol to obtain a broader range of bioactive compounds. In addition, previous studies have only focused on qualitative and quantitative analyses of flavonoid metabolites (Zhang *et*

al., 2022), with no studies on other secondary metabolites. Therefore, targeted qualitative analysis techniques are required to identify the chemical components of bitter gourd and maximise the utilisation of different parts of bitter gourd plants. The findings of this study will contribute to establishing a metabolite profile and provide valuable insights for future isolation and pharmaceutical applications of *M. charantia*.

II. MATERIALS AND METHOD

A. Plant Collection and Extraction

The fresh plant sample (leaves, fruits and seeds) of *M. charantia* was collected in Tawau, Sabah. The species was identified based on morphological characteristics using standard references (Flora of Peninsular Malaysia, Flora of Java), and the identification was verified by botanists at Universiti Malaysia Terengganu (UMT).

The leaves, fruits and seeds were cleaned under running tap water and dried at room temperature for seven days before being ground into powder form using a grinder. The plant powders were extracted by soaking them with three different solvents, 500 mL of Hexane (95%), 500 mL ethyl acetate (95%) and 500 mL methanol (95%) at room temperature for 72 h. Then, each extract was concentrated using a rotary evaporator (Büchi Rotavap R-200CH-9230, Switzerland) under reduced pressure at 35 °C–40 °C for 15 min. The crude extracts obtained were weighed and stored in a chiller (4 °C) for further analysis.

B. Preliminary Phytochemical Screening

All crude extracts were subjected to nine phytochemical tests which were alkaloids, saponins, tannin (Braymer's test) (Morsi, 2014), steroids (Yelin & Kuntadi, 2019), flavonoids (H_2SO_4 test), phlobatannins (Coolborn *et al.*, 2015), reducing sugar test (Fehling's test) (Karki, 2018), carbohydrate (iodine test) (Karki, 2018) and phenolic content test (ferric chloride test) (Pant *et al.*, 2017).

C. Scavenging Effect on DPPH Radical

The DPPH free radical scavenging assay was done using the methodology described by Gülçin *et al.* (2010). About 1.0 mL of each plant extract was mixed with 0.25 mL 0.2 mM DPPH radical in a methanol solution. The mixture was shaken vigorously and left to stand for 30 minutes at room temperature. Reduction of DPPH and BHT were also used at 20 mM (in methanol) as a control. The scavenging effect on DPPH radical (%) was calculated using the following formula:

$$Scavenging\ (\%) = \frac{(Absorbance\ of\ the\ control - Ansorbance\ of\ the\ treated\ sample}{Absorbance\ of\ the\ control} \times 100$$

D. Determination of Tocopherol Content

Under dim light and on ice, 0.15 g of fresh sample was ground with 1.5 mL of acetone and clean sand using a mortar and pestle at 0-4 °C. The mixture was then centrifuged at 10,000 rpm at 0-4 °C. The supernatant obtained was extracted with 0.5 mL of hexane, followed by vortex for 30 seconds. The resulting mixture was centrifuged at 10,000 rpm for 10 minutes, after which the top layer was carefully removed. The hexane extraction was repeated twice to ensure efficient compound recovery.

The assay mixture was prepared following the method described by Kanno & Yamauchi (1977). The hexane extract was mixed with 0.4 mL of 0.1% (w/v) PDT (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine dissolved in ethanol) and 0.4 mL of 0.1% ferric chloride (prepared in ethanol). The total volume was adjusted to 3.0 mL with absolute ethanol, and the mixture was gently swirled and allowed to develop colour for 4 minutes. Subsequently, 0.2 mL of orthophosphoric acid was added, and the mixture was left at room temperature for 30 minutes before measuring the absorbance at 554 nm. A blank sample was prepared using the same procedure, except absolute ethanol was used instead of the hexane extract. A standard curve was generated using α-tocopherol (Sigma Type V) at concentrations ranging from 0 to 1.4 μg/mL, and the α-tocopherol content in the leaf, fruit and seed samples was calculated based on this standard curve.

E. Determination of Carotenoid Content

Carotenoid content was estimated following the method described by Lichtenthaler and Buschmann (2005). Under dim light and on ice, 0.02 g of leaf, fruit and seed samples

were ground with 3.0 mL of 80% (v/v) acetone using a mortar and pestle. The homogenate was then centrifuged at 10,000 rpm for 10 minutes, and the absorbance of the resulting supernatant was measured at 663.2 nm, 646.8 nm, and 470 nm. 80% acetone was used as a blank for the spectrophotometric analysis. Carotenoid content was calculated using the formula:

$$Ca = 12.25A_{663.2} - 2.79A_{646.8}$$

$$Cb = 21.50A_{646.8} - 5.10A_{663.2}$$

$$Cx+c = \frac{1000_{470} - 1.82Ca - 85.02Cb}{198}$$

where;

Ca = chlorophyll a (mg/l)

Cb = chlorophyll b (mg/l)

Cx + c = carotenoid (mg/l)

F. Statistical Analysis

The experiment was conducted in 3 replicates (n=3), and data were presented as mean ± standard deviation. The significant differences were determined using a one-way analysis of variance (ANOVA) and tested by Duncan's Multiple Range Test (DMRT) to compare the differences between treatments at a 0.05% significance level.

III. RESULT AND DISCUSSION

A. Percentage Yield of Extracts

The extraction yield of crude extracts from different parts of *M. charantia* (leaves, fruits, and seeds) varied significantly based on the solvent polarity used. Methanol, a highly polar solvent, consistently yielded the highest extract percentage across all plant parts, with the leaves yielding the highest values of 11.05 ± 0.35%, followed by fruits at 8.10 ± 0.16%, and the lowest was observed in seeds at 4.68 ± 0.057% (Table 1). The results indicated that polar solvents like methanol are more effective at extracting polar secondary metabolites, such as flavonoids, phenolics, and alkaloids, which are known to possess antioxidant and medicinal properties (Abdel-Hameed *et al.*, 2013).

In contrast, hexane and ethyl acetate, which are less polar, resulted in lower yields, particularly in the extraction of non-polar and semi-polar compounds, such as lipids and terpenoids (Pradhan *et al.*, 2018). The leaves of *M. charantia* exhibited the highest extraction yield, suggesting that they contain the highest concentration of bioactive compounds, particularly those with polar characteristics (Mitra *et al.*, 2016). These results align with previous studies showing that leaves are often the most phytochemically rich part of medicinal plants (Jadhav *et al.*, 2018).

Similar patterns have been reported in other members of the Cucurbitaceae family. For instance, *Cucurbita pepo* (pumpkin) leaves extracted with methanol demonstrated higher yields and stronger antioxidant activity compared to fruits and seeds, confirming the abundance of polar phytochemicals in leafy tissues (El-Mosallamy *et al.*, 2016). Likewise, *Lagenaria siceraria* (bottle gourd) exhibited higher extraction yields from leaves and shoots than from fruits when methanol was used, again highlighting the solvent's efficiency in recovering polar compounds (Saha *et al.*, 2011). In *Trichosanthes cucumerina* (snake gourd), methanol extracts of leaves were also found to contain rich phenolic and flavonoid content, correlating with strong antioxidant potential (Nayak *et al.*, 2014).

While seeds of *M. charantia* produced the lowest yield, the ethyl acetate extracts showed relatively higher yields than the other parts, indicating the presence of semi-polar compounds that are more efficiently extracted using this solvent. This trend is also consistent with reports on *Citrullus colocynthis* (bitter apple), where seed extractions yielded higher levels of semi-polar cucurbitacins in ethyl acetate compared to methanol (Rahimi *et al.*, 2019). Taken together, these comparisons suggest that methanol is generally the most suitable solvent for extracting bioactive compounds from Cucurbitaceae plants, particularly from leaves, which consistently exhibit the highest potential for therapeutic applications.

Table 1. The weight and percentage yield of crude extracts in different parts of *M. charantia* (Values are means ± SD, n=3).

Plant Parts	Solvent	Yield of extracts (g)	Percentage Yield (%)
Leaf	Hexane	1.92 ± 0.046	7.06 ± 0.57
	Ethyl acetate	0.46 ± 0.016	1.85 ± 0.018

	Methanol	2.75 ± 0.14	11.05 ± 0.35
Fruit	Hexane	0.90 ± 0.025	3.61 ± 0.11
	Ethyl acetate	0.38 ± 0.013	1.50 ± 0.061
	Methanol	2.14 ± 0.12	8.10 ± 0.16
Seed	Hexane	0.27 ± 0.008	1.09 ± 0.026
	Ethyl acetate	0.98 ± 0.036	3.84 ± 0.24
	Methanol	1.83 ± 0.046	4.68 ± 0.057

B. Qualitative Phytochemical Screening Test

The overall results of the qualitative phytochemical screening revealed that the methanol crude extract of *M. charantia* leaves, fruits and seeds contained alkaloids, flavonoids, phenols, tannins, saponins, carbohydrates, reducing sugars, and steroids. In contrast, the ethyl acetate crude extract was found to contain alkaloids, saponins, steroids, carbohydrates, and reducing sugars. The hexane crude extract, on the other hand, showed positive results for alkaloids, steroids, carbohydrates, and reducing sugars. This study also observed that all solvents used did not manage to extract phlobatannin in all parts of the plants. A summary of the phytochemical tests conducted on all crude extracts is provided in Table 2.

Alkaloids represent about 20% of known secondary metabolites in plants (Kaushik *et al.*, 2021). Derived from amino acids, alkaloids hold a distinct place among natural molecules such as sugars, proteins, and lipids (Bhatla & Lal, 2023; Naji *et al.*, 2024). The bioactive compounds are essential for the survival of various organisms and exhibit a wide range of beneficial properties, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and hepatoprotective effects (Riaz *et al.*, 2023; Pereira *et al.*, 2023). In this study, alkaloids have been detected in the leaves, fruits, and seeds of all *M. charantia* extracts. Comparable results were found in the study of Shubha *et al.* (2018); Ingle & Kapgatte (2018) and Mahwish *et al.* (2018), where alkaloids were detected in leaves, fruits and seeds of *M. charantia* These bioactive compounds have been extensively studied for their potential to eliminate and reduce human cancer cell lines. A similar observation was also observed in the leaves of *M. charantia* from India (Leelaprakash *et al.*, 2011), and in the fruits of the same species studied by Mariselvi & Manimegalai (2017). Although specific alkaloids in *M. charantia* are less extensively characterized compared to other metabolite classes, studies have reported several nitrogen-containing compounds with potential bioactivity (Jia *et al.*, 2017). These alkaloids may contribute to antimicrobial and anti-inflammatory properties, as observed in related Cucurbitaceae species.

Flavonoids, a diverse group of plant secondary metabolites, play an essential role in the biological functions of bitter gourd. They are known for their antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic, and antioxidant properties (Shen *et al.*, 2022). In this study, flavonoids were only present in the methanolic crude extract, consistent with previous research by Supraja and Usha (2013). Tannins, which form irreversible complexes with proline-rich proteins and inhibit protein synthesis (Shimada, 2006), were also detected in the methanol extract of *M. charantia*. Our findings are consistent with recent studies that confirmed the presence of tannins in methanol or ethanol extracts of *M. charantia* leaves. For example, Abdullah *et al.* (2024) reported significant total tannin content in ethanolic leaf extracts of *M. charantia*, alongside notable levels of phenolic and flavonoid compounds. Similarly, Abdul Rahim *et al.* (2021) demonstrated that methanol was among the most effective solvents for extracting phenolic metabolites, including tannins, from *M. charantia* fruits. These results reinforce the role of polar solvents in recovering polyphenolic compounds. However, contrasting evidence exists, as Thakre *et al.* (2014) found no detectable tannins in methanol extracts of *M. charantia* fruits, suggesting that tannin content may vary depending on plant part, solvent system, and growing conditions.

In this study, methanol extracts were particularly rich in flavonoids and phenolic acids. Identified flavonoids include quercetin, kaempferol, luteolin, and apigenin derivatives, while phenolic acids such as gallic acid, caffeic acid, and chlorogenic acid have also been reported (Bara *et al.*, 2025; Jia *et al.*, 2017). These compounds are well known for their antioxidant, anti-inflammatory, and cardioprotective properties. Their abundance in methanol extracts correlates with the strong DPPH radical scavenging activity observed in this study.

Table 2. Qualitative phytochemical constituents of *Momordica charantia*

Screening Test	Extracts*								
	Leaves			Fruits			Seeds		
	H	EA	M	H	EA	M	H	EA	M
Alkaloids	+	+	+	+	+	+	+	+	+
Flavonoid	-	-	+	-	-	+	-	-	+
Steroids	+	+	+	+	+	+	+	+	+
Saponins	-	+	+	-	+	+	-	+	+
Tannin	-	-	+	-	-	+	-	-	+
Phlobatannin	-	-	-	-	-	-	-	-	-
Phenolic content	-	-	+	-	-	+	-	-	+
Carbohydrate	+	+	+	+	+	+	+	+	+
Reducing sugar	+	+	+	+	+	+	+	+	+

**H: Hexane, EA: Ethyl Acetate, M: Methanol*

Saponins, well-known bioactive phytochemicals with antioxidant, antimicrobial, cytotoxic, anti-inflammatory, and immunostimulatory activities (Francis *et al.*, 2002), were detected in all crude extracts tested. While methanol efficiently recovered saponins in the present study, previous reports have also documented their extraction in ethyl acetate and other semi-polar solvents, depending on the plant matrix and tissue type (Anjamma & Bhavani, 2015; Jia *et al.*, 2017; Sparg *et al.*, 2004). This solvent-dependent distribution reflects the amphiphilic nature of saponins, which contain both hydrophilic glycoside moieties and hydrophobic aglycone backbones, allowing them to partition across polar and semi-polar solvents.

The presence of steroids in all crude extracts, with confirmation given by the characteristic greenish-blue coloration after treatment with concentrated sulfuric acid, consistent with the report of Supraja and Usha (2013). Although methanol is a highly polar solvent and thus suitable for dissolving a broad range of terpenoids, it is not exclusive in this regard. Terpenoids have also been effectively extracted using solvents of lower polarity, such as hexane and ethyl acetate, reflecting the chemical diversity within this large class of metabolites (Tiwari *et al.*, 2011; Santos-Sánchez *et al.*, 2019). Hence, the efficiency of extraction is better understood in terms of solvent polarity and selectivity rather than the use of a single solvent.

It is also important to note that, although steroids are classified as a subclass of terpenoids due to their shared tetracyclic ring skeleton, they can be distinguished by specific structural modifications. Steroids typically possess characteristic alkyl side-chain substitutions at C-17 and variations in hydroxylation patterns, whereas other terpenoids, such as monoterpenes and sesquiterpenes, differ in chain length, degree of unsaturation, and oxygenation (Mahato & Sen, 1997). These structural differences not only influence their solubility in different solvents but also underpin their diverse biological functions.

Steroidal saponins and cucurbitane type triterpenoids represent the signature bioactive group in *M. charantia*. Among these, charantin (a sterol glucoside mixture) is one of the most cited markers and is often used to standardise bitter gourd extracts due to its hypoglycaemic activity (Jia *et al.*, 2017). In addition, over 80 cucurbitane type triterpenoids have been identified, including momordicosides (A, B, C, F1, G, K, L) and momordicins, many of which exhibit antidiabetic, anticancer, and immunomodulatory effects (Fatope *et al.*, 2004; Harinantenaina *et al.*, 2006). The predominance of these triterpenoids in methanol extracts reflects their polar to semi-polar nature, which is efficiently extracted by highly polar solvents.

Carbohydrates, essential energy sources for living organisms (Adair, 2007; Jiang *et al.*, 2014), were detected in all crude extracts of *M. charantia*. Our findings corroborate the study by Anjamma & Bhavani (2015), who reported carbohydrates in the fruits and leaves. The presence of reducing sugars, as indicated by Fehling's solution, was observed in all extracts, in agreement with the study by Joseph & Jini (2013). However, no phlobatannins were detected in any of the crude extracts, consistent with the research by Supraja & Usha (2013), which found negative results in hexane extracts. In contrast, phlobatannins were present in the ethanolic extract of bitter gourd studied by Gayathry and John (2022). Lastly, phenolic compounds were present in all methanol crude extracts, which aligns with the findings of Anjamma & Bhavani (2015) and Jia *et al.* (2017), confirming the presence of phenolic constituents in the leaves, fruits, and seeds of *M. charantia*. Based on the above results, methanolic extract was selected as the best

solvent to further study the antioxidative properties of *M. charantia*.

Collectively, the presence of diverse metabolite classes such as alkaloids, flavonoids, phenolic acids, saponins, triterpenoids, proteins, nucleosides, and antioxidant micronutrients underscore the therapeutic potential of this species. Nevertheless, the relative abundance of these compounds is strongly influenced by the solvent system and plant part used, emphasising the need for targeted quantification (HPLC or LC–MS/MS) in future studies on Malaysian cultivars.

C. Free Radical Scavenging Assays

The antioxidant activity of the methanolic extracts of *M. charantia* was evaluated using the DPPH radical scavenging assay, which measures the ability of compounds to neutralise free radicals. As shown in Figure 2, the leaves exhibited the highest scavenging activity at 98.43 ± 0.09%, significantly outperforming both the fruits (87.61 ± 0.30%) and seeds (71.26 ± 1.00%). These results suggest that the antioxidant potential of *M. charantia* is part-dependent, with the leaves being the most effective at scavenging DPPH free radicals. For comparison, the positive control butylated hydroxytoluene (BHT) exhibited 98.70 ± 0.10% scavenging activity, indicating that the antioxidant capacity of *M. charantia* leaves is comparable to a standard synthetic antioxidant.

The observed differences in scavenging activity between the plant parts imply that phytochemicals soluble in methanol are primarily responsible for the antioxidant effects, with the leaves containing the highest concentration of bioactive compounds with radical-scavenging properties. Methanol, being a polar solvent, is highly effective in extracting polar antioxidant compounds, such as phenolics, flavonoids, and alkaloids, which are well-documented for their ability to neutralise free radicals (Rao *et al*., 2013). The lower scavenging activity observed in the fruits and seeds suggests that these parts either contain fewer antioxidant compounds or may require different extraction strategies to release their bioactive metabolites.

Overall, the findings highlight the strong antioxidant potential of *M. charantia* leaves, which perform nearly as effectively as BHT. This reinforces their potential for therapeutic and nutraceutical applications in combating oxidative stress-related diseases, while also supporting the use of natural plant-derived antioxidants as promising alternatives to synthetic compounds.

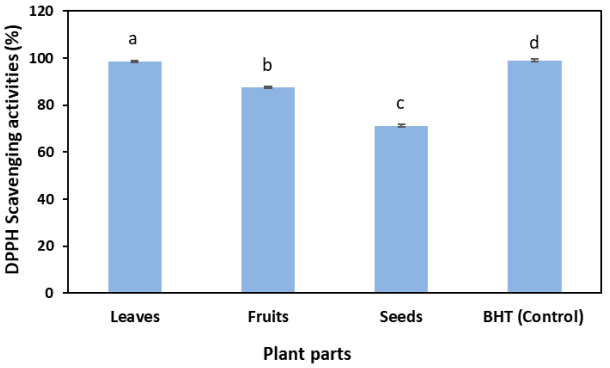

Figure 2. DPPH Scavenging activities (%) in the methanolic crude extract of *M. charantia*. (Values are means ± SD, n=3). Bars with different letters (a - d) indicate significant differences at $p < 0.05$.

D. Non-enzymatic Antioxidant Assays

Tocopherol is a lipid-soluble antioxidant that plays a crucial role in neutralising reactive oxygen species (ROS) and lipid radicals, thereby protecting cellular structures from oxidative damage. It is primarily localised in the thylakoid membrane of chloroplasts, where it contributes to the stability of photosynthetic membranes and prevents lipid peroxidation. Among the different isomers of tocopherol, α-tocopherol is reported to exhibit the highest antioxidative potential due to its unique isomeric structure, which allows it to act as an effective chain-breaking antioxidant (Mesa & Munné-Bosch, 2023). The ability of α-tocopherol to donate hydrogen atoms stabilises free radicals and interrupts the oxidative chain reactions that lead to cell membrane damage (Kumar *et al.*, 2020).

In this study, the α-tocopherol concentration in different parts of *M. charantia* was determined, as shown in Figure 3. Among the three plant parts analyzed, the leaves exhibited the highest α-tocopherol content (4.65 ± 0.01 mg/g.fwt), followed by the fruits (1.95 ± 0.12 mg/g.fwt), while the seeds had the lowest concentration (0.12 ± 0.02 mg/g.fwt). The higher accumulation of α-tocopherol in the leaves may be attributed to their role in photosynthesis, where they require enhanced protection against oxidative stress caused by light

exposure. The relatively lower levels in the fruits and seeds suggest that these plant parts may rely on alternative antioxidant mechanisms to counteract oxidative damage. These findings align with previous studies, which indicate that α-tocopherol levels are typically highest in green, photosynthetically active tissues, where oxidative stress is more pronounced (Szewczyk *et al.*, 2021).

The variation in α-tocopherol content across different parts of *M. charantia* highlights the plant's ability to regulate antioxidant distribution based on physiological functions. The high concentration in leaves suggests a protective role in safeguarding chloroplast membranes against photooxidative stress, while the moderate presence in fruits may contribute to fruit maturation and seed development. Comparable findings have been reported in other members of the Cucurbitaceae family. For instance, *Cucurbita pepo* (zucchini) fruits were shown to contain α-tocopherol concentrations ranging between 1.2–3.5 mg/100 g fresh weight, depending on cultivar and maturity stage (Kostecka-Gugała *et al.*, 2015). Similarly, *Cucumis sativus* (cucumber) exhibited α-tocopherol levels of 0.8–2.1 mg/100 g fresh weight in fruit tissues (El-Massry *et al.*, 2020). In pumpkin (*Cucurbita maxima*), α-tocopherol concentrations in pulp were reported at 3.4–5.9 mg/100 g fresh weight, while seeds contained much higher values, up to 39.5 mg/100 g oil (Murkovic *et al.*, 2002). These comparisons indicate that the α-tocopherol content of *M. charantia* leaves is relatively high within the Cucurbitaceae family, reinforcing its potential as a natural dietary source of vitamin E.

However, the significantly lower concentrations in seeds imply that other antioxidants, such as flavonoids or carotenoids, may play a more dominant role in seed protection. These findings underscore the importance of α-tocopherol in plant defence mechanisms and suggest that the leaves of *M. charantia* could be a valuable source of natural antioxidants for pharmaceutical and nutraceutical applications.

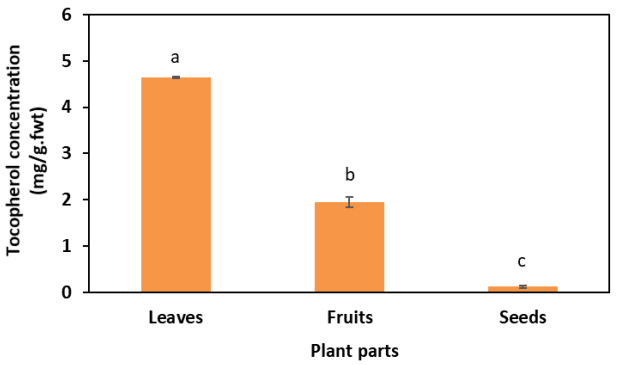

Figure 3. Tocopherol concentrations (mg/g.fwt) in the methanolic crude extract of *M. charantia*. (Values are means ± SD, n=3). Bars with different letters (a - c) indicate significant differences at $p < 0.05$.

Carotenoids are the most widely distributed pigments in nature and are present in photosynthetic bacteria, some species of archaea and fungi, algae, plants, and animals. These pigments play a crucial role in photosynthesis by absorbing light energy and protecting plant tissues from oxidative stress through their antioxidant properties (Sun *et al.*, 2022). Carotenoids are classified into two main groups: carotenes (such as β-carotene and α-carotene) and xanthophylls (such as lutein, zeaxanthin, and violaxanthin). Approximately 70 different carotenoid compounds and their derivatives have been identified in various fruits and vegetables, with β-carotene being one of the most well-known and widely distributed among them. β-Carotene is particularly significant due to its role as a precursor to vitamin A, which is essential for human health, including vision, immune function, and skin health (Maoka, 2019).

The highest carotenoid concentration was found in the leaves (44.18 ± 1.81 mg/g.fwt), followed by the fruits (32.72 ± 3.09 mg/g.fwt) and seeds (24.28 ± 2.79 mg/g.fwt) as shown in Figure 4. The greater accumulation of carotenoids in the leaves may be attributed to their direct involvement in photosynthesis, where they function as accessory pigments that capture light energy and protect chlorophyll from photooxidative damage. Among the various carotenoids, violaxanthin is one of the predominant pigments in green leaves, contributing to the xanthophyll cycle, which plays a key role in photoprotection and energy dissipation under high light conditions (Lichtenthaler, 2009). The moderate levels of carotenoids in the fruits suggest their role in attracting pollinators and seed dispersers, as well as

providing antioxidant protection during fruit development and ripening. The presence of carotenoids in the seeds, albeit in lower concentrations, indicates their potential role in protecting seed tissues from oxidative stress, thereby ensuring seed viability and germination.

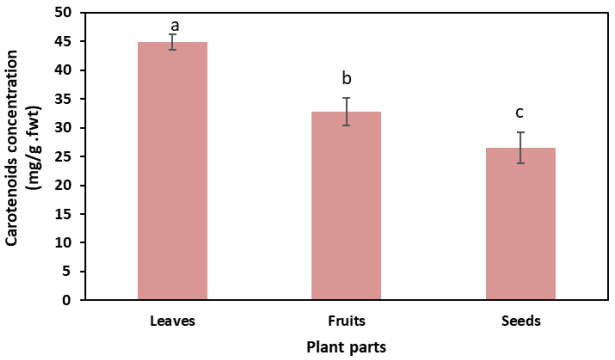

Figure 4. Carotenoid concentrations (mg/g.fwt) in the methanolic crude extract of *M. charantia*. (Values are means ± SD, n=3). Bars with different letters (a–c) indicate significant differences at $p < 0.05$.

The findings of this study highlight the differential distribution of carotenoids in *M. charantia*, emphasising the importance of these pigments in plant physiology and their potential health benefits. Given their strong antioxidant properties, carotenoids from *M. charantia* could be explored as natural bioactive compounds in pharmaceutical and nutraceutical applications. The high carotenoid content in the leaves, in particular, suggests their potential as a valuable dietary source of these beneficial compounds. Further studies focusing on the identification and quantification of specific carotenoid compounds in *M. charantia* could provide deeper insights into their functional properties and potential industrial applications.

IV. CONCLUSION

Phytochemical screening and antioxidant assays were successfully conducted on *Momordica charantia* (bitter gourd). Various parts of the *M. charantia* plant (leaves, fruits, and seeds) were extracted using different solvent polarities, namely hexane, ethyl acetate, and methanol. The results demonstrated that the methanol crude extract contained a diverse range of secondary metabolites, with percentage yields of 11.05 ± 0.35% for the leaves, 8.10 ± 0.16% for the fruits, and 4.68 ± 0.057% for the seeds. The phytochemical tests revealed that the methanol crude extract of the leaves exhibited positive results for all tested phytochemical groups. For the antioxidant potential, the methanol extract of the leaves demonstrated the highest DPPH free radical scavenging activity, α-tocopherol and carotenoids, further supporting the leaves' superior antioxidant capacity.

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