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Phytochemical screening and total antioxidant potential of *Momordica charantia* extract

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Abstract

Medicinal plants, known for their accessibility and cost-effectiveness, have a historical track record as therapeutic sources. Today, there's increasing awareness regarding the overuse and misuse of synthetic drugs. Efforts worldwide aim to explore and document ethnomedicinal wisdom and scientific research on medicinal plants. Throughout history, plants and herbal remedies have served as medicinal solutions, and recent research has validated many traditional claims about their therapeutic properties. This study aimed to measure the total phenolic and flavonoid content and evaluate antioxidant activity in *Momordica* extracts. Soxhlet extraction was employed to prepare the extracts, resulting in varying yields. Qualitative phytochemical analysis identified alkaloids, saponins, flavonoids, tannins, and glycosides in the extracts. Among the solvents used, aqueous extracts displayed the highest phenolic and flavonoid content and significant DPPH radical scavenging activity. In conclusion, *Momordica* extracts represent abundant sources of natural antioxidants, with their properties varying depending on the solvent employed.

Keywords: *Momordica*, flavonoid, phenolic, DPPH

Introduction

Medicinal plants have long been recognized as readily available and cost-effective sources of therapeutic agents throughout history. In recent times, there is growing public awareness of the over-prescription and misuse of synthetic medications (Cowan 1999) [6]. Globally, approximately 7,500 medicinal plants have been utilized for treating various ailments. This has sparked a rising interest worldwide in researching and analyzing plant-based products for their potential health benefits. Efforts are being made across the world to explore and document ethnomedicinal knowledge and scientific research related to medicinal plants (Ganie and Yadav 2014) [10]. Throughout history, plants and herbal remedies have served as medicinal solutions. Recent research conducted over the past few decades has substantiated numerous traditional claims regarding the therapeutic properties of various plants. India's rich biodiversity features numerous plant species renowned for their medicinal attributes (Jain, Khatana *et al.* 2019) [12]. Ancient practices of using various parts of these plants to address health concerns have a long history. Purohit and Vyas estimated that around 70,000 plant species have been employed as herbal remedies at different times. Medicinal plants, abundant in phenolic compounds, provide a range of biological effects, including their role as antioxidants. These antioxidants can augment our natural defense mechanisms, protecting cells from oxidative damage caused by free radicals (Dwivedi 2007) [9].

Momordica charantia, commonly known as Bitter Melon or Karela, is a tropical vegetable found in Indian cuisine and valued in folk medicine for its potential in managing diabetes. The Latin name "*Momordica*" refers to the leaf's jagged edges, resembling bite marks. In Ayurveda, this fruit is considered a tonic, stomachic, stimulant, emetic, antibilious, laxative, and alterative. Bitter melon has a history of use in various Asian traditional medicine systems. Its bitterness can aid digestion, benefiting those with sluggish digestion, dyspepsia and constipation. Although it may worsen heartburn and ulcers in some cases, its demulcent and mild inflammation-modulating properties often mitigate these effects, as reported in traditional use and clinical experience (Kumar, Sharathnath *et al.* 2010) [17]. The major chemical constituents of MC include hetero polysaccharides (galactose, glucose, arabinose, rhamnose,

and mannose), proteins and peptides (e.g., momordins, momorcharins, MAP30, and MC lectin, which are part of the ribosome-inactivating proteins family), terpenoids, saponins (Cucurbitanes and cucurbitacines), flavonoids, phenolic compounds, and various other compounds like essential oils, fatty acids, amino acids, and sterols (Anilkumar *et al.* 2010; Schrot, Weng *et al.* 2015, Dandawate, Subramaniam *et al.* 2016) [2, 30, 7]. Numerous phytochemicals, including momorcharins, momordicin, charantin, cucurbitacins, and diosgenin, have been isolated from bitter melon (Murakami, Emoto *et al.* 2001) [24].

Lifestyle and dietary habits contribute to chronic low-grade inflammation, impacting immune function and gut microbiota. Dietary components have potential in modulating chronic inflammatory conditions and aiding in their treatment, although much remains unclear about their specific anti-inflammatory mechanisms (Minihane, Vinoy *et al.* 2015) [23]. *Momordica charantia* (MC) dietary supplementation has been extensively studied for conditions like T2DM, dyslipidemia, obesity, and cancer, highlighting its potential for lowering blood sugar and lipids. However, clinical trials have yielded inconclusive results (Alam, Uddin *et al.* 2015) [1]. MC extracts were found to inhibit inflammation by targeting the NF- κ B pathway. They reduced TNF- α production and the expression of inflammatory genes in RAW 264.7 cells while decreasing NF- κ B activity and phosphorylation of various signaling molecules (Renushe *et al.* 2022; Kobori, Ohnishi-Kameyama *et al.* 2008) [29, 16]. These extracts also lowered NO and prostaglandin E2 production and suppressed inducible NO synthase and IL-1 β expression (Lii, Chen *et al.* 2009) [21]. In addition, MC extract demonstrated dose-dependent inhibition of NO production and downregulated ERKs and Akt, reducing NF- κ B and AP-1 activity (Svobodova, Barros *et al.* 2017; Kumar and Reddy, 2012) [32, 20]. In a clinical trial, water-soluble bitter melon fruit extract significantly reduced blood glucose levels in NIDDM diabetics during OGTT. Bitter melon leaf extracts (water, ethanol, methanol) displayed broad-spectrum antimicrobial activity (Khan and Omoloso 1998) [14]. MC and its phytochemicals, including alpha and beta-momorcharin, lectin, and MAP 30, exhibited *in vitro* antiviral effects against various viruses, including HIV, Epstein-Barr, herpes, coxsackievirus B3, and polio. Notably, MAP 30, a 30 kDa protein, demonstrated promising anti-HIV activity, while alpha momorcharin displayed abortifacient, tumor suppressive, and anti-HIV properties (Ng, Chan *et al.* 1992) [25].

Materials and methods

Various chemicals, including DPPH, Quercetin, Gallic acid, and others from Sigma Chemicals. Importantly, all chemicals used were of analytical grade.

Fresh *Momordica* were washed, shade-dried for 40-45 days at room temperature, powdered, and then subjected to Soxhlet extraction using solvents of increasing polarity: petroleum ether, benzene, chloroform, acetone, hexane, and aqueous solution (Deyab, Elkatomy *et al.* 2016) [8]. The resulting extract was concentrated, dried, and stored in brown bottles at room temperature. These extracts were later analyzed for various secondary metabolites, including phenols, flavonoids, tannins, saponins, alkaloids, glycosides, phytosterols, steroids, and carbohydrates (Khan, Shah *et al.* 2016) [15].

The total phenolic content in leaf extracts was determined using the Folin Ciocalteu method with Gallic acid (25-200 μ g/ml) as the calibration standard, expressed as Gallic acid equivalents (GAE) per gram of extracts. In a 96-well plate, 50

μ l of the extract (1 mg/ml) or standard solution were mixed with 50 μ l of distilled water, 50 μ l of 10% Folin Ciocalteu phenol reagent, and 50 μ l of 1M sodium carbonate solution. After a 60-minute incubation at room temperature, shielded from light, absorbance was measured at 750nm using a microplate reader. Total phenolic content was expressed as μ g GAE per ml of plant extracts (Sembiring, Elya *et al.* 2018) [31]. Total flavonoid content in *Ficus religiosa* leaf extracts was determined using the aluminum chloride method with Quercetin (25-200 μ g/ml) as the standard, expressed as Quercetin equivalents (QE) per gram of extract. After mixing 50 μ l of extract (1 mg/ml) or standard solution with 10 μ l of 10% aluminum chloride, 150 μ l of 95% ethanol, and 10 μ l of 1M sodium acetate in a 96-well plate, the mixture was incubated for 40 minutes at room temperature, shielded from light. Absorbance was measured at 415nm using a microplate reader, and total flavonoid content was expressed as μ g QE per ml of plant extracts (Sembiring, Elya *et al.* 2018, Aparna, Madhuri *et al.* 2021) [31, 3].

The DPPH radical scavenging activity of various extracts was assessed following the Jose Prieto method with slight modifications. In a 96-well plate, we combined 20 μ l of extract stock solution (1.075 to 200 μ g/ml) with 180 μ l of DPPH solution (0.147mM). After a 30-minute incubation at room temperature in darkness, we measured absorbance at 517nm using a microplate reader. Methanol served as the blank, and ascorbic acid acted as the positive standard. These tests were conducted in triplicate, and IC50 values (the concentration causing 50% DPPH inhibition) were calculated (Prieto 2012) [27].

Results and Discussion

Momordica extracts yielded between 3.49 and 12.07 g per 50 g of leaves, with the highest in aqueous and the lowest in chloroform. Phytochemical screening identified alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, saponins, and steroids in all extracts. *Momordica* extracts showed varying total flavonoid content, ranging from 61.84 to 265.47 μ g Quercetin equivalent (QE) per milligram. Among these, the highest content was in the aqueous extract (265.47 μ g QE/mg), followed by benzene (143.98 μ g QE/mg), hexane (84.65 μ g QE/mg), petroleum ether (63.94 μ g QE/mg), chloroform (61.84 μ g QE/mg), and acetone (45.68 μ g QE/mg). The phenolic content in *Momordica* extract varied from 29.55 to 43.35 μ g Gallic Acid Equivalents (GAE) per milligram. The highest phenolic content was found in aqueous extracts (43.35 μ g GAE/mg), followed by benzene (41.68 μ g GAE/mg), acetone (40.25 μ g GAE/mg), petroleum ether (36.47 μ g GAE/mg), chloroform (34.56 μ g GAE/mg), and hexane (29.55 μ g GAE/mg). IC50 values, indicating the concentration at which the extracts neutralized 50% of DPPH radicals, were determined using the DPPH free radical scavenging method, with ascorbic acid as the standard. The inhibitory activity, observed within 30 minutes, for *Momordica* extracts and ascorbic acid, at varying concentrations (ranging from 10.49 to 12.13 μ g/ml), ranged from approximately 44.38% to 49.78%.

Table 1: Extractive yield of different extracts of *Momordica*

Solvent	Extractive yield (g/50g)
Acetone	3.7
Aqueous	12.07
Benzene	9.0
Petroleum Ether	3.57
Chloroform	3.49
Hexane	3.83

Table 2: Qualitative phytochemical screening of different extracts of *Momordica*

Phytochemical Constituent	Aqueous	Acetone	Benzene	Chloroform	Hexane	Petroleum Ether
Alkaloids	-	+	+	-	+	+
Glycosides	+	+	-	-	-	+
Flavanoids	+	+	+	+	+	+
Tannins	-	-	-	+	-	-
Carbohydrate	+	-	-	-	+	-
Proteins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Triterpenoids	+	-	-	+	-	+
Steroids	-	-	-	-	-	+
Saponnins	-	+	-	+	-	-

Table 3: Quantification of total flavonoid and total phenolic content in different extracts of *Momordica*

Name of the extract	Total flavonoid content ($\mu\text{g QE/mg extract}$)	Total phenolic content ($\mu\text{g GAE/mg extract}$)
Aqueous	265.47	43.35
Acetone	45.68	40.25
Benzene	143.98	41.68
Chloroform	61.84	34.56
Hexane	84.65	29.55
Petroleum Ether	63.94	36.47

Table 4: DPPH radical scavenging activity of *Momordica* extract

Extract	IC50 ($\mu\text{g/ml}$)
Ascorbic acid	10.87
Acetone	12.11
Aqueous	10.49
Benzene	12.13
Chloroform	10.88
Hexane	10.54
Petroleum ether	10.68

Flavonoids and phenolic compounds, naturally occurring in various plant parts, serve as antioxidants, known for their free radical scavenging and antioxidant properties due to their reducing and chelating abilities. Table 3 summarizes the phenolic and flavonoid content in *Momordica* extract, with the highest total phenolic content (TPC) recorded at 43.35 $\mu\text{g/mg GAE}$ and a significant flavonoid content (TFC) of 265.47 $\mu\text{g/mg QE}$, highlighting its quality. Flavonoids, influenced by environmental, genetic and seasonal factors, contribute to disease defense (Kumar and Roy 2018) [19]. Plant foods rich in flavonoids, such as flavonols, play a crucial role in protecting against conditions like coronary heart disease and dementia. Rutin, a potent plant-based antioxidant known as quercetin-3-rutinoside, offers various health benefits, including managing diabetes, oxidative stress, microbial infections, cancer, and cardiovascular issues (Rauf, Imran *et al.* 2017) [28]. *Momordica* exhibits variable values for total phenolic content (TPC) and total flavonoid content (TFC), with the highest yield in aqueous extract and the lowest in hexane and acetone. Aqueous and Benzene extracts of *Momordica* yield the most. Phytochemical analysis identified alkaloids, flavonoids, glycosides, phenols, tannins, and terpenoids in various extracts, while steroids and tannins were absent in all ether extracts. These findings are consistent with previous studies that also found similar compounds in *Ficus religiosa* leaf extracts (Ghadigaonkar, Reddy *et al.* 2021) [11]. *Momordica* is a potent antioxidant source, with aqueous extracts containing the highest total flavonoid content, followed by benzene, hexane, petroleum ether, chloroform and acetone extracts (Pal, Sharma *et al.* 2018) [26]. Aqueous extracts of *Momordica* also exhibited the highest total phenolic content, followed by benzene, acetone, petroleum ether, chloroform and hexane extracts. All *Momordica*

extracts demonstrated significant, dose-dependent DPPH scavenging activity, indicating their ability to neutralize reactive oxygen species (Balakrishnan, Shrivastava *et al.* 2014, Maimonaparveen, Madhuri *et al.* 2021) [4, 22]. The benzene extract showed the highest DPPH radical scavenging activity among all extracts (Jyothi, Reddy *et al.* 2009, Charde, Dhongade *et al.* 2010) [13, 5].

Conclusion

Phytochemicals are gaining recognition for their unique therapeutic properties and applications in human and animal health. This study emphasizes the antioxidant potential of *Momordica* extracts, demonstrating their ability to inhibit the generation of free radicals *in vitro* through significant dose-dependent DPPH scavenging activity. The aqueous extract exhibited the highest DPPH scavenging activity, with phenolic compounds playing a key role in antioxidant action and protection against oxidative stress. This suggests that *Momordica* holds promise as a therapeutic agent for various ailments, while also validating the traditional use of *Momordica* in herbal medicine.

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