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Phytochemical analysis and total antioxidant potential of *Moringa oleifera* leaf extract

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Abstract
This study aimed to quantify total phenolic and flavonoid content and assess antioxidant activity in leaf extracts of *Moringa oleifera*. Extracts were prepared using Soxhlet extraction, yielding various amounts. Qualitative phytochemical analysis revealed alkaloids, saponins, flavonoids, tannins and glycosides in the extracts. Aqueous extracts showed the highest phenolic and flavonoid content. They exhibited substantial DPPH radical scavenging activity. Overall, *Moringa oleifera* extracts proved to be rich sources of natural antioxidants with varying properties among different solvents.

Keywords: Phenolic, flavonoid, DPPH, *Moringa oleifera*, phytochemical

Introduction
Throughout ancient human history, plants have played a significant role in a wide range of applications. One of the earliest documented instances of plant use for medicinal purposes dates back around 5000 years ago, as evidenced by a Sumerian clay tablet discovered in Nagpur. Moreover, historical records from civilizations such as Mesopotamia, Egypt, Greece, and the Islamic world also shed light on the extensive utilization of plants for various purposes (Petrovska 2012) [18]. In contemporary times, there has been a noticeable global shift from synthetic medicines to a preference for herbal remedies, often described as a "return to nature." It's important to recognise that plant-based medications have been integral to the progression of human healthcare for millennia.

A study was conducted to determine the potential benefits of *Moringa oleifera* (MO), also known as the drumstick tree or horseradish tree, which originated in Northern India and Africa. (Leone, Spada *et al*. 2016) [14]. The leaves of the *Moringa* tree, which are the most commonly utilized part, are highly nutritious, containing vitamins, carotenoids, protein, iron, and potassium (Verma, Vijayakumar *et al*. 2009) [24]. Additionally, *Moringa* leaves are rich in bioactive compounds, particularly polyphenols like phenolic acids and flavonoids, as well as four unique *Moringa* isothiocyanates, known for their strong biological activities (Waterman, Rojas-Silva *et al*. 2015) [25]. These leaves can be consumed fresh, cooked, or stored as a dried powder for extended periods without refrigeration, with minimal loss of nutritional value. Due to these qualities, *Moringa oleifera* leaf (MOL) has been explored for its potential in treating various diseases, including cardiovascular issues, insulin resistance, hepatic steatosis, and more (Verma, Vijayakumar *et al*. 2009) [24]. *Moringa oleifera* leaves have demonstrated their protective potential for spermatogonial cells and in alleviating cell damage induced by cyclophosphamide in mice (Nayak, Honguntikar *et al*. 2016) [40]. The presence of flavonoids and isothiocyanates in *Moringa* leaves has been linked to beneficial effects on chronic diseases, including the suppression of NF-κB and PI3K/Akt signaling pathways (Renushe *et al*. 2022; Kou, Li *et al*. 2018) [12, 22]. Furthermore, In diabetic rats, *Moringa oleifera* was found to mitigate nephrotoxic and hepatotoxic damage induced by STZ by exhibiting anti-apoptotic and anti-inflammatory effects. There is evidence that extracts from fresh *Moringa oleifera* leaves can reduce edema induced by carrageenan in hindpaws (Gupta, Jain *et al*. 2018) [28]. Additionally, various extracts, such as aqueous, 80% methanol, and 70% ethanol extracts of freeze-dried *Moringa* leaves, have shown antioxidant activities. The plant has a documented history of diverse health benefits, including anti-inflammatory, antimicrobial, antidepressant, anti-hyperlipidemic, anti-ulcer, and anti-fertility properties. (Gupta, Jain *et al*. 2018) [8].

Materials and Methods

Various chemicals, including 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 3-(2-pyridyl)-5, 6-diphenyl-12, 4-triazine-4,4-disulfonic acid, potassium ferricyanide, Quercetin, Gallic acid, ascorbic acid, Folin –Ciocalteu phenol reagent, and sodium carbonate, were procured from Sigma Chemicals. It's worth noting that all the chemicals utilized in the study were of analytical grade.

In order to dry fresh *Moringa oleifera* leaves, they were washed twice with distilled water followed by 40-45 days of shade drying at room temperature. A mechanical blender was used to powder the dried leaves, and then Soxhlet extraction was conducted. A sequence of solvents of increasing polarity was used for extraction, including petroleum ether, benzene, chloroform, acetone, hexane, and finally, aqueous solution. (Deyab, Elkatony *et al*. 2016) [4]. Following extraction, the resulting extract was concentrated using a flash evaporation evaporator, with solvent recovery, and subsequently dried in desiccators. The dried extracts were stored in brown bottles at room temperature.The crude leaf extracts were analyzed to determine a range of secondary metabolites, including phenols, flavonoids, tannins, saponins, alkaloids, glycosides, phytosterols, steroids, and carbohydrates (Khan, Shah *et al*. 2016) [11].

The total phenolic content in leaf extracts was determined using the Folin Ciocalteu method with slight modifications. Gallic acid (25-200 ug/ml) served as the standard for calibration, and the results were expressed as Gallic acid equivalents (GAE) per gram of extracts. Standard solutions of Gallic acid ranging from 1.56 to 100ug/ml were prepared in water. In a 96-well plate, 50ul of the extract (1mg/ml) or standard solution were mixed with 50ul of distilled water, followed by the addition of 50ul of 10% Folin Ciocalteu phenol reagent and 50ul of 1M sodium carbonate solution. A blank with distilled water was included. After incubating the reactions for 60 minutes at room temperature, shielded from light, the absorbance was measured at 750nm using a microplate reader. The total phenolic content was expressed as ug GAE per ml of plant extracts (Sembiring, Elya *et al*. 2018) [23].

The total flavonoid content in various *Moringa oleifera* leaf extracts was determined using the aluminum chloride method. Quercetin (25-200 ug/ml) was employed as a standard, and the results were expressed as Quercetin equivalents (QE) per gram of extract. Standard Quercetin solutions with concentrations ranging from 1.56 to 100 ug/ml were prepared in 80% ethanol. In a 96-well plate, 50 ul of the extract (1 mg/ml) or standard solution was mixed with 10 ul of 10% aluminum chloride solution, followed by the addition of 150ul of 95% ethanol and 10ul of 1M sodium acetate. An 80% ethanol reagent blank was included. After incubating the mixture for 40 minutes at room temperature, shielded from light, the absorbance was measured at 415nm using a microplate reader. Total flavonoid content was expressed as ug Quercetin equivalents (QE) per ml of plant extracts (Aparna *et al*. 2021; Sembiring, Elya *et al*. 2018) [1, 23].

DPPH radical scavenging activity of various extracts was assessed using the Jose Prieto method, with slight modifications. The antioxidant activity of each extract was evaluated in a 96-well plate. In each well, 20 ul of extract stock solution at different concentrations (Ranging from 1.075 to 200 ug/ml) was mixed with 180ul of DPPH solution (0.147 mM). After 30 minutes of incubation at room temperature in a dark environment, the absorbance was measured at 517nm using a microplate reader. Methanol served as the blank, and ascorbic acid was used as the positive standard. All tests were conducted in triplicate, and the IC50 value (the concentration resulting in 50% inhibition on DPPH) was calculated (Prieto 2012) [19].

Results and Discussion

The extract yields from *Moringa oleifera* leaves varied between 0.88-12.15 g per 50 g of leaves, with the highest yield observed in acetone and the lowest in petroleum ether. Phytochemical screening of the extracts identified the presence of alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids saponnins and steroids in all leaf extracts. However, carbohydrates and proteins were absent in extracts. The total flavonoid content in *Moringa oleifera* leaf extracts varied from 17.42 to 145.25 µg Quercetin equivalent (QE) per milligram of extract. Among the six crude extracts, the highest total flavonoid content was found in the aqueous extract (145.25 µg QE/mg), followed by acetone (101.39 µg QE/mg), benzene (61.93 µg QE/mg), hexane (28.13 µg QE/mg), petroleum ether (19.12 µg QE/mg), and chloroform (17.42 µg QE/mg) from *Moringa oleifera* leaves. The phenolic content in *Moringa oleifera* leaf extract was quantified as µg GAE equivalent per milligram of extract. Among the extracts, total phenolic content ranged from 9.57 to 72.31 µg Gallic Acid Equivalents (GAE) per milligram. Aqueous extracts exhibited the highest phenolic content at 72.31 µg GAE/mg, followed by acetone (33.26 µg GAE/mg), petroleum ether (23.57 µg GAE/mg), chloroform (19.23 µg GAE/mg), hexane (17.42 µg GAE/mg), and benzene (9.57 µg GAE/mg) from *Moringa oleifera* leaves. The IC50 values, representing the concentration at which extracts scavenge 50% of DPPH radicals, were determined using the DPPH free radical scavenging method with ascorbic acid as the standard. The inhibitory activity (at 30 min) of *Moringa oleifera* leaf extracts and ascorbic acid, all at different concentrations (ranging from 10.11 to 11.21 µg/ml), was found to be around 45.49% to 48.47%.

Table 1: Extractive yield of different extracts of *Moringa oleifera* leaf

Solvent	Extractive yield (g/50g)
Acetone	12.15
Aqueous	8.8
Benzene	1.44
Petroleum Ether	0.88
Chloroform	3.71
Hexane	6.05

Table 2: Qualitative phytochemical screening of different extracts of *Moringa oleifera* leaf

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 *Moringa oleifera* leaf:

Name of the extract	Total flavonoid content (µg QE/mg extract)	Total phenolic content (µg GAE/mg extract)
Aqueous	145.25	72.31
Acetone	101.39	33.26
Benzene	61.93	9.57
Chloroform	17.42	19.23
Hexane	28.13	17.42
Petroleum Ether	19.12	23.57

Table 4: DPPH radical scavenging activity *of Moringa oleifera* leaf extract

Extract	IC50(µg/ml)
Ascorbic acid	11.21
Acetone	11.01
Aqueous	10.52
Benzene	11.06
Chloroform	10.27
Hexane	10.88
Petoleum ether	10.11

Flavonoids and phenolic compounds, found in various plant parts, act as natural antioxidants. They are known for their ability to scavenge free radicals and exhibit antioxidant properties, attributed to their reducing and chelating abilities. Table 1 summarizes the phenolic, flavonoid content in *Moringa oleifera* leaf extract. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, with the *Moringa oleifera* extract exhibiting the highest TPC at 72.31 µg/mg GAE. Flavonoids play a vital role in disease defense (Rajanandh and Kavitha 2010) [20], with a TFC of 145.25 µg/ mg QE in this extract, reflecting its high-quality flavonoid content. Environmental and genetic factors, as well as seasonal variations, influence flavonoid concentrations (Kumar and Roy 2018) [13]. Plant foods rich in flavonoids, like flavonols, play a crucial role in protecting against conditions like coronary heart disease and dementia (Fang, Tang *et al*. 2013) [6]. Rutin, also known as quercetin-3-rutinoside, is a potent plant-based antioxidant with various health benefits, including managing diabetes, oxidative stress, microbial infections, cancer, and cardiovascular issues (Rauf, Imran *et al*. 2017) [21]. Different studies reported varying values for total phenolic content (TPC) and total flavonoid content (TFC) in *Moringa oleifera*, demonstrating some variability in these measurements. *Moringa oleifera* exhibited the highest yield in acetone extract, with the lowest in petroleum ether. Acetone and aqueous extracts of *Moringa oleifera* leaves had the highest yields. Phytochemical analysis showed the presence of alkaloids, flavonoids, glycosides, phenols, tannins and terpenoids in various leaf extracts, while steroids and glycosides were absent in acetone and petroleum ether extracts. These findings align with previous studies that also identified phenolic compounds, tannins, sterols, saponins, terpenoids, and glycosides in *Ficus religiosa* leaf extracts (Ghadigaonkar, Reddy *et al*. 2021) [7]. *Moringa oleifera* served as an antioxidant source, and this study highlighted its rich antioxidant potential. Among the six *Moringa oleifera* extracts, aqueous extracts contained the highest total flavonoid content, followed by acetone, benzene, hexane, petroleum ether, and chloroform extracts (Pal, Sharma *et al*. 2018). Aqueous extracts of *Moringa oleifera* also exhibited the highest total phenolic content, with acetone, petroleum ether, chloroform, hexane, and benzene extracts following in descending order. Reactive oxygen species like superoxide, hydroxyl radicals and hydrogen peroxide are byproducts of biological reactions and can harm cells when overproduced. All *Ficus religiosa* leaf extracts exhibited significant, dose-dependent DPPH scavenging activity (Maimonaparveen *et al*. 2021; Balakrishnan, Shrivastava *et al*. 2014) [15, 2]. The DPPH method, which employs a stable free radical system, is a sensitive means of assessing *in vitro* antioxidant activity in plant extracts. The extracts of FR demonstrated the ability to donate hydrogen to free radicals, neutralizing the electron responsible for radical reactions. The highest DPPH radical scavenging activity was observed in the aqueous extract, surpassing all other extracts (Jyothi *et al*, 2010; Charde, Dhongade *et al*. 2010) [10, 3].

Conclusion

Phytochemicals are gaining significant recognition due to their unique therapeutic attributes and extensive applications in human and animal well-being. The study highlights the antioxidant potential of *Moringa oleifera* leaf extracts by inhibiting the generation of free radicals *in vitro*. These extracts showed significant dose-dependent DPPH scavenging activity, indicating their ability to neutralize stable free radicals. The highest DPPH scavenging activity was observed in the aqueous extract. Phenolic compounds, particularly in the aqueous extract, contributed to the antioxidant action and protection against oxidative stress, making *Moringa oleifera* a potential therapeutic agent for various ailments. Overall, this study validates the use of *Moringa oleifera* leaves in traditional medicine.

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