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# In Vitro Antioxidant Potential of Moringa oleifera Flower, Leaf, and Pod Soup

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**Abstract:** Antioxidants are essential in combating oxidative stress and supporting overall health. This study investigates the in vitro antioxidant potential of soups prepared using Moringa oleifera flowers, leaves, and pods by analyzing their nutrient composition, phytochemical content, and antioxidant activity to evaluate their functional food potential. Two soup variations were formulated with different proportions: Variation 1 contained 10 g of flowers, 5g of leaves, and 15 g of pods (10:5:15), while Variation 2 consisted of 15 g of flowers, 5 g of leaves, and 10 g of pods (15:5:10). Proximate composition was analyzed using AOAC methods, phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) identified key bioactive compounds, and antioxidant activity was evaluated using DPPH and FRAP assays. Sensory attributes were assessed using a 9-point hedonic scale by 50 semi-trained panelists. Results revealed that Variation 1 exhibited higher DPPH radical scavenging activity and greater levels of total phenolics, flavonoids, and anthocyanins, whereas Variation 2 showed stronger ferric-reducing antioxidant power and a more diverse range of bioactive constituents. Sensory evaluation favored Variation 2 for its enhanced appearance, flavor, aroma, and overall acceptability. In conclusion, both Moringa soup variations offer significant antioxidant and nutritional benefits, highlighting Moringa oleifera's potential as a functional food ingredient that promotes health while catering to different consumer preferences.

**Keywords:** In vitro analysis, Moringa oleifera, soup, antioxidant, oxidative stress, phytochemicals, GC-MS, DPPH, FRAP, sensory evaluation.

## I. INTRODUCTION

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is a key factor in the development of chronic diseases such as cardiovascular disorders, diabetes, neurodegenerative conditions, and cancer [1], [2], [3], [4]. ROS can damage mitochondrial DNA, disrupt enzymatic function and cellular signaling, and induce cell death, with lifestyle factors further aggravating these effects.

While ROS and reactive nitrogen species (RNS) support physiological processes at normal levels, their excess leads to oxidative and nitrosative stress, damaging cellular components and promoting inflammation, aging, and cancer [5], [6], [7], [8]. Although the body possesses endogenous antioxidant systems, dietary antioxidants have gained attention, especially with the rise of the free radical theory of aging [9].

Moringa oleifera, known as the "miracle tree," is valued for its adaptability and medicinal properties [10], [11].

Traditionally used to treat various ailments, its parts are rich in bioactive compounds with antioxidant, anti-inflammatory, and nutritional benefits [12], [13], [14], [15], [16], [17].

Given its phytochemical richness, M. oleifera shows potential in dietary strategies to combat oxidative stress and support health.

## II. REVIEW OF LITERATURE

### A. Oxidative Stress and Diseases

Oxidative stress is a condition characterized by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, leading to damage of proteins, lipids, carbohydrates, and DNA [18]. It can originate from both internal sources, such as mitochondrial activity and inflammation, and external factors like pollution, radiation, smoking, and poor diet. Prolonged oxidative stress is closely associated with the onset of chronic diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions [19].

### B. Antioxidants–An Overview

Antioxidants are compounds that prevent or delay oxidative damage by stabilizing free radicals through electron donation [20]. They help maintain redox balance and protect cellular structures. The body employs enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (e.g., vitamins C and E, flavonoids, selenium) antioxidants. Given the limitations of endogenous defenses under oxidative stress, dietary intake of antioxidant-rich foods is essential. Natural sources, particularly plant-based functional foods, are gaining attention for their health-promoting potential [21].

### C. Free Radicals and Their Biological Impact

Free radicals are unstable molecules with unpaired electrons that readily interact with and damage cellular components, including lipids, proteins, and DNA. ROS such as hydroxyl radicals, superoxide anions, and hydrogen peroxide are byproducts of normal metabolism, particularly in mitochondria, but can increase under stress. Endogenous sources include mitochondrial respiration and enzymatic reactions, while external contributors include UV radiation, toxins, and smoking. Their accumulation contributes to aging and disease progression, underscoring the importance of antioxidant defenses [22].

### D. *Moringa oleifera*–A Natural Source of Antioxidants

*Moringa oleifera*, also known as the "miracle tree," is a drought-resistant plant native to South Asia and widely cultivated in tropical regions [23]. It is valued for its nutritional content and therapeutic uses, particularly in low-resource settings. The leaves are rich in vitamins A, C, and E, and minerals such as calcium, iron, and potassium [24], [25]. Pods are high in dietary fiber and protein. *Moringa* also contains various phytochemicals like flavonoids, phenolic acids, and glucosinolates, which contribute to its antioxidant properties [26].

### E. Therapeutic and Antioxidant Potential and Safety Considerations

*Moringa* has demonstrated significant antioxidant potential, with its leaves and flowers exhibiting high levels of phenolics and flavonoids, outperforming many vegetables in antioxidant assays such as DPPH, FRAP, ABTS, and ORAC [27], [28]. It also supports immune and metabolic health, as shown in animal studies [29]. Additionally, *Moringa* is used in green synthesis of nanoparticles with anti-inflammatory and anticancer properties [30], [31].

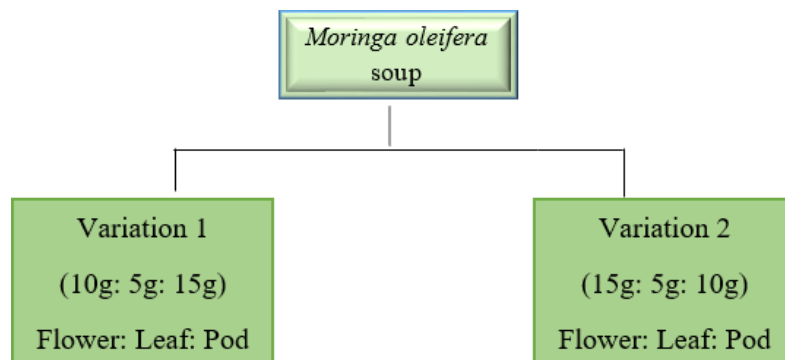
Toxicological evaluations indicate general safety at moderate intake levels. Aqueous leaf extracts showed no toxicity up to 2000 mg/kg in rats [32], while higher concentrations of methanolic extracts require caution [33]. This study complied with Food Safety and Standards Regulations (2016), using 10–20 g of flowers and leaves, and 40–80 g of pods per formulation, within recommended limits.

## III. RESEARCH METHODOLOGY

An *in vitro* experimental research design was employed to evaluate the antioxidant potential of the infused drink. The laboratory analyses for the present study were carried out at Affyclone Laboratories Pvt. Ltd., an ISO-certified facility located in Chromepet, Chennai. Sensory evaluation was conducted in the Food Science Laboratory, Department of Home Science, Women's Christian College, Chennai. Ethical clearance for the study was obtained from the Institutional Ethics Committee of the Department of Home Science, Women's Christian College, Chennai.

### A. Procurement and preparation of the *Moringa* soup

*Moringa oleifera* flowers, leaves, and pods were freshly procured from a local home garden and thoroughly washed under running water to remove dirt and other impurities. Two variations of *Moringa* soup were prepared using different proportions of the plant parts. In Variation 1, the flower, leaf, and pod components were used in the ratio of 10:5:15 grams, respectively. These ingredients were combined with 30 grams of tomato, 30 grams of onion, half a teaspoon each of pepper, cumin, and salt, and a pinch of turmeric. The mixture was pressure-cooked for three whistles, cooled, and blended into a fine purée. A pinch of pepper powder was added before serving. This variation contained a higher proportion of pods compared to flowers and leaves. In Variation 2, the flower, leaf, and pod components were used in the ratio of 15:5:10 grams, respectively. The same additional ingredients and cooking procedure were followed. After pressure cooking and blending, the soup was similarly seasoned with a pinch of pepper powder. This variation had a higher proportion of flowers relative to the leaves and pods.


Fig.1 Variations of the *Moringa oleifera* soup

### B. Preparation of extracts

The aqueous and ethanol extracts from both variations of the *Moringa oleifera* flower, leaf, and pod soup were prepared for comprehensive phytochemical and antioxidant analyses. For the aqueous extract, the soups were brought to a gentle boil and then filtered using Whatman filter paper to remove solid residues. The resulting clear filtrates were collected in sterile containers and used for nutrient quantification, phytochemical screening, and *in vitro* antioxidant assays. For the ethanol extract, equal volumes of ethanol were added to the soup samples in a 1:1 ratio at room temperature. The mixtures were thoroughly stirred and left to stand for 24 hours to facilitate the extraction of ethanol-soluble bioactive compounds. After the extraction period, the solutions were filtered to remove particulate matter. The ethanol extracts were subsequently analyzed using Gas Chromatography– Mass Spectrometry (GC-MS) to identify phytoconstituents [34].

### C. Assessment of *in vitro* antioxidant potential of the soup

The *in vitro* antioxidant potential of both variations of the *Moringa oleifera* soup was evaluated using two well-established assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP). Each assay was conducted using standard protocols across a concentration range of 20  $\mu$ L to 100  $\mu$ L, and the IC<sub>50</sub> values representing the concentration required to achieve 50% activity were determined for each sample.

The DPPH assay is a widely accepted method for assessing the free radical scavenging ability of plant-based products. It relies on the ability of antioxidants present in the sample to donate electrons or hydrogen atoms to the stable DPPH radical, resulting in a visible color shift from deep violet to yellow. This decolorization, which corresponds to a decrease in absorbance at 517 nm, is directly proportional to the antioxidant capacity of the sample. The simplicity, rapidity, and reproducibility of the DPPH assay make it a preferred choice for evaluating natural antioxidants in food and nutraceutical [35].

In contrast, the FRAP assay measures the sample's capacity to reduce ferric ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ) ions under acidic conditions, forming a blue-colored  $\text{Fe}^{2+}$ -TPTZ complex. The intensity of the resulting color, quantified by absorbance at 593 nm, reflects the electron-donating capacity or reducing power of the antioxidants in the sample. This method, though limited to compounds that act via single electron transfer, remains a valuable and cost-effective tool for comparing the antioxidant strength of different samples, particularly in the context of functional food development [36].

### D. Data analysis

The data collected from the study were analyzed to determine the mean, standard deviation, and correlation using Microsoft Excel.

## IV. RESULTS AND DISCUSSION

The results of the nutrient analysis for Variation 1 and Variation 2 of the *Moringa oleifera* flower, leaf, and pod soup are presented in Table I.

TABLE I

MICRONUTRIENT AND MACRONUTRIENT CONTENT OF THE MORINGA SOUP

Nutrients	Unit	Variation 1	Variation 2
Carbohydrates	g/100ml	0.8673	4.897



Fat	Percent/100 ml	0.355	0.36
VitaminA	mg/100 ml	22	22
VitaminC	mg/100 ml	202.7397	145.8447
Iron	mg/100 ml	10.125	12.5
Selenium	mg/100 ml	22.216	20.1

This specific nutrient composition analysis of the two variations of *Moringa oleifera* soup highlighted key differences in their macronutrient and micronutrient content. Variation 2 emerged as the superior formulation due to its enhanced carbohydrate and calcium content, making it a better energy source with improved mineral availability. Although the protein content showed only a slight difference between the two variations, both remained relatively low. The fat content was consistent across both formulations, indicating that ingredient modifications did not impact the lipid profile. Vitamin A levels were higher in Variation 1, suggesting a potential difference in ingredient retention during preparation. Iron content, though slightly reduced in Variation 2, remained within a beneficial range. These findings suggest that Variation 2 offers a more balanced nutritional profile, making it a more favorable option for individuals seeking an energy-dense and mineral-rich diet.

#### A. Identification of bioactive compounds using GC-MS

The Gas Chromatography–Mass Spectrometry (GC-MS) results for both variations of the infused *Moringa oleifera* drink are presented in Tables III and IV. The analysis revealed a broad spectrum of bioactive compounds classified into various phytochemical groups, including phenols, flavonoids, lactones, fatty acids, glycosides, triterpenoids, steroids, and alkaloids. Several compounds were identified in both variations, such as 2,3-Butanediol [R-(R,R)], Glyceraldehyde, Dihydroxyacetone, Isosorbide Dinitrate, 2(3H)-Furanone, dihydro-4-hydroxy-, 1,2,3-Propanetriol, 1-acetate, Acetoxyacetic acid, nonyl ester,  $\alpha$ -D- Galactopyranoside, methyl, Melezitose, and 4-O-Methylmannose.

Bioactive compounds uniquely present in Variation 1 included Propane, 1-isothiocyanato-; Butanoic acid, 2-methyl-3-oxo-, ethylester; 6-Hydroxy-2,6-dihydropyran-3-one; Pentanoic acid, 4-oxo-; 2(3H)-Furanone, 5-ethyl dihydro-; 2-Isopropyl oxan-4-ol; Phenol, 3,5-bis(1,1-dimethylethyl)-[37]; Heptasiloxane, hexadecamethyl-[38]; and others such as Agaricic acid and Acetamide derivatives. These compounds are reported to possess antioxidant, antibacterial, anticancer, neuroprotective, hypoglycaemic, and anti-inflammatory properties [39], [40], [41].

Variation 2, on the other hand, was characterized by compounds including 5-Oxotetrahydrofuran-2-carboxylic acid; 2-(Isobutoxymethyl)oxirane; Melibiose; 1-Deoxy-d-mannitol; Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-[38]; 5,14,23-Octadecatrien-14,15-diol; and 1H-Benzimidazole, 2-benzyl-1-isobutyl-. These compounds also exhibit antioxidant, antimicrobial, and metabolic-enhancing effects [42], [43], [44], although their therapeutic breadth appears more limited compared to those in Variation 1.

Overall, the findings indicate that Variation 1 exhibits a more comprehensive and potent profile of bioactive constituents with established antioxidant and pharmacological activities. Therefore, Variation 1 may offer superior protection against oxidative stress and related pathologies such as cardiovascular diseases, neurodegenerative disorders, cancers, and diabetes mellitus [45], [46].

#### B. Quantification of phytochemicals

The phytochemical composition of the two *Moringa oleifera* soup variations is detailed in Table II. Variation 1, containing 10 g of flowers, 5 g of leaves, and 15 g of pods, demonstrated notably higher levels of total phenolic content ( $47.452 \pm 3.219$  mg GAE/100 ml) and total flavonoids ( $26.904 \pm 8.454$  mg QE/100 ml) compared to Variation 2 (15 g flowers, 5 g leaves, 10 g pods), which recorded lower values of phenolics ( $33.616 \pm 21.575$  mg GAE/100 ml) and flavonoids ( $21.587 \pm 10.183$  mg QE/100 ml). Interestingly, Variation 2 exhibited a greater total antioxidant capacity ( $558 \pm 4.588$  mg AAE/100 ml) than Variation 1 ( $491.684 \pm 19.601$  mg AAE/100 ml).

These findings indicate that while Variation 2 may contain certain compounds with stronger immediate radical-scavenging activity, the higher levels of phenolic and flavonoid compounds in Variation 1 suggest a more robust and sustained antioxidant potential. Research shows that these compounds play a crucial role in providing antioxidant, anti-inflammatory, and therapeutic benefits, contributing to the functional properties of the formulation [47], [48].

TABLE II  
QUANTIFICATION OF PHYTOCHEMICALS IN THE MORINGA SOUP

Phytochemicals	Variation 1	Variation 2
Total phenolic content (mgGAE/100 ml)	47.452±3.219	33.616±21.575
Total flavonoids (mgQE/100ml)	26.904±8.454	21.587±10.183
Anthocyanin content (mgCGE/100ml)	491.684± 19.601	558±4.588
Total antioxidant content (mgAAE/100 ml)	47.452±3.219	33.616±21.575

### C. *In vitro* Antioxidant potential of the infused drinks

To determine the antioxidant capacity of the infused drinks, both DPPH and FRAP assays were employed. The DPPH assay revealed a concentration-dependent increase in radical scavenging activity for both variations. However, at sample volumes ranging from 20  $\mu$ l to 100  $\mu$ l, the antioxidant response was relatively modest. The IC<sub>50</sub> values were recalculated to better understand their effectiveness—237.9  $\mu$ l for Variation 1 and 216.88  $\mu$ l for Variation 2. As the IC<sub>50</sub> represents the volume needed to neutralize 50% of DPPH radicals, the lower value in Variation 2 indicates a higher antioxidant efficiency at comparable concentrations. Despite both variations demonstrating activity, the results suggest that Variation 2 is marginally more effective in combating oxidative stress through radical scavenging. The outcomes of the DPPH assay are depicted in Figure 2.

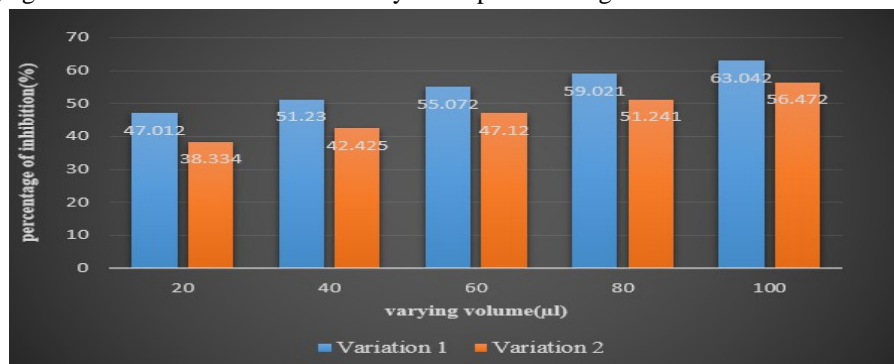


Fig. 2 Percentage of Inhibition of Variation 1 and Variation 2 of the Moringa soup

For further analysis, the FRAP assay was conducted to evaluate the ferric ion reducing ability of the samples. Both variations demonstrated a progressive increase in reducing power with increasing concentrations, indicating a positive correlation between concentration and antioxidant activity. The IC<sub>50</sub> values derived from the FRAP data were 39.44  $\mu$ l for Variation 2 and 46.64  $\mu$ l for Variation 1, implying that Variation 2 exhibits greater reducing capability by achieving 50% of maximum activity at a lower dose. These findings suggest that Variation 2 possesses a stronger ferric reducing antioxidant potential, which may be attributed to the presence of active compounds that function efficiently at lower concentrations. The FRAP assay results are illustrated in Figure 3.

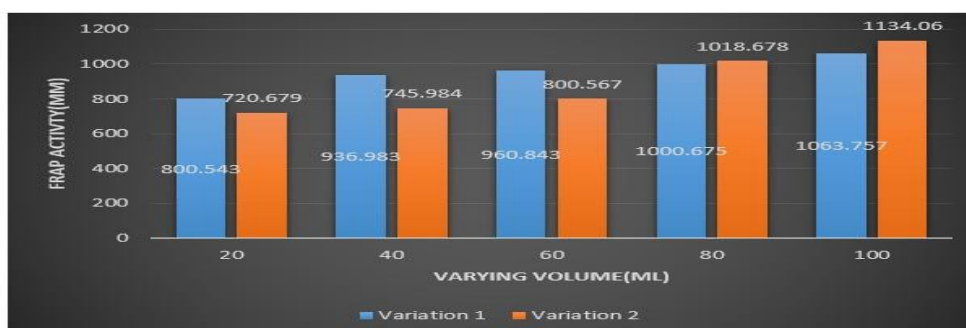


Fig. 3 FRAP Activity of Variation 1 and Variation 2 of the Moringa soup

## V. CONCLUSION

Based on the results from nutrient analysis and antioxidant assays (total antioxidant capacity, DPPH, and FRAP), Variation 1 exhibited superior radical scavenging activity compared to Variation 2, whereas Variation 2 demonstrated significantly greater ferric reducing power. Consequently, both *Moringa* soup variations possess considerable antioxidant potential, with Variation 2 showing a slightly enhanced profile. Furthermore, sensory evaluation revealed that Variation 2 outperformed Variation 1 in terms of appearance, flavor, color, odor, and overall acceptability. Therefore, the ultimate choice of *Moringa* soup formulation may be guided by individual consumer preferences.

## VI. ACKNOWLEDGMENT

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