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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 Moringasoupvariations offersignificantant ioxidantandnutritionalbenefits, highlightingMoringaoleifera'spotential as a functional food ingredient that promotes health while catering to different consumer preferences.

Keywords: Invitroanalysis, Moringaoleifera, soup, antioxidant, oxidativestress, 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].ROScandamage mitochondrialDNA,disruptenzymaticfunctionandcellularsignaling,andinduce 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 oxidativeandnitrosativestress,damagingcellularcomponents 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 richin bio active compounds with antioxidant, anti-inflammatory, and benefits [12], [13], [14], [15], [16], [17].

Given its phytochemical richness, M. oleifer a shows potential indicatory strategies to combatoxidative stress and support health.

II. REVIEW OF LITERATURE

A. OxidativeStressandDiseases

Oxidative stress is a condition characterized byan imbalance between reactive oxygen species (ROS) and the body's antioxidantdefense 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].



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B. Antioxidants-AnOverview

Antioxidants are compounds that prevent or delay oxidative damage by stabilizing free radicals through electron donation[20].Theyhelp maintainredoxbalanceandprotectcellularstructures.Thebodyemploysenzymatic(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. FreeRadicalsandTheirBiologicalImpact

Freeradicalsareunstablemoleculeswithunpairedelectronsthatreadilyinteractwithanddamagecellularcomponents, 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. Moringaoleifera-ANaturalSourceofAntioxidants

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. Theleaves are richinvitamins A,C, and E, and mineral 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. TherapeuticandAntioxidantPotentialandSafetyConsiderations

Moringa has demonstrated significant antioxidant potential, with its leaves and flowers exhibiting high levels of phenolicsandflavonoids, 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 upto2000mg/kginrats[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 laboratoryanalysesforthepresentstudywerecarriedoutat AffycloneLaboratoriesPvt.Ltd.,anISO-certifiedfacilitylocatedin Chromepet, Chennai. SensoryevaluationwasconductedintheFoodScienceLaboratory,DepartmentofHomeScience,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. ProcurementandpreparationoftheMoringasoup

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. Thesameadditionalingredientsandcooking procedurewerefollowed. Afterpressurecookingandblending,thesoupwas similarly seasoned with a pinch of pepper powder. This variation had a higher proportion of flowers relative to the leaves andpods.

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Fig.1Variationsofthe Moringaoleifer a soup

B. Preparationofextracts

Theaqueousandethanolextractsfrombothvariationsofthe *Moringaoleifera*flower,leaf,andpodsoupwereprepared for comprehensive phytochemical and antioxidantanalyses.For theaqueousextract,thesoups werebroughtto agentleboiland thenfilteredusing Whatman filterpapertoremovesolidresidues.Theresultingclearfiltrateswerecollectedinsterilecontainers and used for nutrient quantification, phytochemical screening, and in vitro antioxidant assays. For the ethanol extract, equal volumesofethanol wereaddedtothesoupsamplesina1:1ratioatroomtemperature.The mixtures werethoroughlystirredand left to stand for 24 hours to facilitate the extraction of ethanol-soluble bioactive compounds. After the extraction period, the solutionswere filteredtore moveparticulatematter.TheethanolextractsweresubsequentlyanalyzedusingGasChromatography– Mass Spectrometry (GC-MS) to identify phytoconstituents [34].

C. Assessmentofinvitroantioxidantpotentialofthe 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 μ L to 100 μ L, and the IC50 values representing the concentration required to achieve 50% activity were determined for each sample.

The DPPH assayis a widelyaccepted 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 (Fe³⁺) to ferrous (Fe²⁺) ions under acidic conditions, formingablue-colored Fe²⁺-TPTZ complex. The intensity of the resulting color, quantified by absorbance at 593nm, reflects the electron-donating capacity or reducing power of the antioxidants in the sample. This method, though limited to compound sthat activity is in the context of functional food development [36].

D. Data analysis

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

IV. RESULTS AND DISCUSSION

Theresultsofthenutrientanalysis for Variation 1 and Variation 2 of the Moringa oleifera flower, leaf, and podsoupare presented in Table I.

TABLE I

MICRONUTRIENTANDMACRONUTRIENTCONTENTOFTHE MORINGASOUP

Nutrients	Unit	Variation 1	Variation2			
Carbohydrates	g/100ml	0.8673	4.897			



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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

Thespecificnutrientcompositionanalysisofthetwovariationsof *Moringaoleifera*souphighlightedkeydifferencesin their macronutrient and micronutrientcontent. Variation2emergedasthesuperiorformulationduetoitsenhancedcarbohydrate 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, indicatingthatingredient modificationsdid notimpactthelipid profile.VitaminAlevels werehigher in Variation 1, suggesting a potential difference in ingredient retention during preparation. Iron content, though slightly reduced in Variation 2, remained withinabeneficialrange. ThesefindingssuggestthatVariation2offersa morebalancednutritionalprofile, making it a more favorable option for individuals seeking an energy-dense and mineral-rich diet.

A. IdentificationofbioactivecompoundsusingGC-MS

TheGasChromatography–MassSpectrometry(GC-MS)resultsforbothvariationsoftheinfused *Moringaoleifera* drinkare 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, α -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;Pentanoicacid,4-oxo-;2(3H)-Furanone,5-ethyldihydro-;2-Isopropyloxan-4-ol;

Phenol,3,5-bis(1,1-dimethylethyl)-[37];Heptasiloxane, hexadecamethyl-[38];and otherssuchas Agaricicacid and Acetamide derivatives.Thesecompoundsarereportedtopossessantioxidant,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-Benzoimidazole, 2-benzyl-1-isobutyl-. These compounds also exhibit antioxidant, antimicrobial,andmetabolic-enhancingeffects[42],[43],[44],althoughtheirtherapeuticbreadthappearsmorelimitedcompared 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 10gofflowers,5gofleaves,and15gofpods,demonstratednotablyhigherlevelsoftotalphenoliccontent($47.452\pm3.219mgGAE/100ml$)andto talflavonoids($26.904\pm8.454mgQE/100ml$)compared to Variation2(15gflowers,5gleaves,10gpods), which recorded lower values of phenolics (33.616 ± 21.575 mgGAE/100 ml) and flavonoids (21.587 ± 10.183 mg QE/100 ml). Interestingly, Variation2exhibitedagreatertotalantioxidantcapacity($558\pm4.588mgAAE/100ml$)thanVariation1($491.684\pm19.601mgAAE/100ml$).

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 playa crucial role in providing antioxidant, anti-inflammatory, and therapeutic benefits, contributing to the functional properties of the formulation [47], [48].



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QUANTIFICATIONOFPHYTOCHEMICALSINTHEMORINGASOUP					
Phytochemicals	Variation1	Variation2			
Totalphenoliccontent (mgGAE/100 ml)	47.452±3.219	33.616±21.575			
Totalflavonoids (mgQE/100ml)	26.904±8.454	21.587±10.183			
Anthocyanincontent (mgCGE/100ml)	491.684± 19.601	558±4.588			
Totalantioxidant content (mgAAE/100 ml)	47.452±3.219	33.616±21.575			

 TABLEII

 QUANTIFICATIONOFPHYTOCHEMICALSINTHE MORINGASOUP

C. InvitroAntioxidantpotentialoftheinfuseddrinks

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 µlto 100 µl, the antioxidant response was relatively modest. The IC50 values were calculated to be the runder stand

theireffectiveness-237.9µlforVariation1and216.88µlforVariation2.AstheIC50representsthevolumeneededtoneutralize 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 Moring as oup to the second second

Forfurtheranalysis, the FRAP assaywasconducted to evaluate the ferricion 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 IC50 values derived from the FRAP data were 39.44 µl for Variation 2 and 46.64 µl for Variation1, implying that Variation2 exhibits greater reducing capability by achieving 50% of maximum activity at a lower dose. These findings suggest that Variation2 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.3FRAPactivityofVariation1andVariation2oftheMoringasoup



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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 Variation1 interms of appearance, flavor, color,odor, andoverall acceptability. Therefore, the ultimate choice of *Moringa* soup formulation may be guided by individual consumer preferences.

VI. ACKNOWLEDGMENT

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