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In Vitro Antioxidant Activity and Phytochemical Correlation of Hydroethanolic Root Extracts from Three Medicinal Plants Assessed by DPPH and ABTS Radical Scavenging

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Abstract- The present study aimed to evaluate the phytochemical composition and in vitro antioxidant potential of hydroethanolic root extracts of Momordica charantia,Ocimum sanctum,and Moringa oleifera.The plant materials were collected,authenticated,shade-dried,and extracted using a 70:30 ethanol–water solvent system by the maceration method.Preliminary phytochemical screening revealed the presence of major bioactive constituents,including alkaloids,flavonoids,phenolics,tannins,saponins,terpenoids,and glycosides in all extracts.

Quantitative analysis demonstrated that Moringa oleifera possessed the highest total phenolic content(91.6±3.2 mg GAE/g extract)and total flavonoid content(67.8±2.7 mg QE/g extract),followed by Ocimum sanctum and Momordica charantia.The antioxidant activity was assessed using DPPH and ABTS radical scavenging assays,where all extracts exhibited concentration-dependent activity.Among the tested samples,Moringa oleifera showed the strongest antioxidant potential with the lowest IC_{50}values in both DPPH(41.3±1.8µg/mL)and ABTS(39.5±1.6µg/mL)assays,followed by Ocimum sanctum and Momordica charantia.

Correlation analysis revealed a strong positive relationship between phytochemical content and antioxidant activity,with total phenolic content showing a high correlation with DPPH(r=0.92)and ABTS(r=0.89)assays,while total flavonoid content also exhibited significant correlations(r=0.90 and 0.87,respectively).These findings indicate that phenolics and flavonoids are the major contributors to the antioxidant activity of the extracts.

Keywords-Antioxidant activity,DPPH assay,ABTS assay,Phytochemicals,Total phenolic content,Total flavonoid content,Hydroethanolic extract,Moringa oleifera.

I. INTRODUCTION

Antioxidants are bioactive molecules capable of inhibiting or delaying the oxidation of different molecules,thereby protective cells,tissues,and biomolecules from oxidative harm as a result of reactive oxygen species(ROS)and free radicals.Oxidation is a regular metabolic approach,however immoderate oxidation can generate ROS,which includes superoxide anion(O_2^-),hydroxyl

radicals(•OH),singlet oxygen($^1O_2$),and hydrogen peroxide(H_2O_2).In addition to exogenous sources such as environmental pollutants,ultraviolet(UV)radiation,tobacco smoke,heavy metals,pesticides,and other pollutants,these reactive species can also arise naturally in the course of cardiocellular metabolism,specifically in mitochondria.

1.1 Classification of Antioxidants

Antioxidants are classified based on their source,mode of action,solubility,and enzymatic nature.Broadly,they are categorized into enzymatic and non-enzymatic antioxidants.Each category has several subtypes with specific roles in protecting cells from oxidative damage.

1. Enzymatic Antioxidants

Enzymatic antioxidants are naturally occurring proteins that catalytically neutralize reactive oxygen species(ROS)and prevent oxidative stress.These enzymes act synergistically in a cascade to detoxify free radicals.

Table.1.1Enzymatic Antioxidants:

Enzyme	Source	Mechanism of Action
Superoxide Dismutase(SOD)	Cytoplasm,mitochondria,extracellular fluids	Converts superoxide radicals(O_2^-)into hydrogen peroxide(H_2O_2)and oxygen(O_2),reducing superoxide toxicity.
Catalase(CAT)	Peroxisomes	Decomposes H_2O_2into water and oxygen,preventing accumulation of $H_2O_2$and formation of hydroxyl radicals.
Glutathione Peroxidase(GPx)	Cytoplasm,mitochondria	Reduces $H_2O_2$and lipid hydroperoxides to water and alcohols using reduced glutathione(GSH)as a cofactor.
Glutathione Reductase(GR)	Cytoplasm	Regenerates reduced glutathione(GSH)from oxidized glutathione (GSSG),maintaining cellular redox balance.

2. Non-Enzymatic Antioxidants

Non-enzymatic antioxidants are low-molecular-weight compounds that directly scavenge free radicals or inhibit oxidative reactions.They are further divided based on source,solubility,and chemical nature.

A. Based on Source

1. Endogenous(Produced by the body)

- Glutathione(GSH):Tripeptide that scavenges ROS and regenerates other antioxidants.
- Coenzyme Q10 (Ubiquinone):Lipid-soluble antioxidant in mitochondria;prevents lipid peroxidation.
- Uric Acid:Acts as a scavenger of hydroxyl and peroxyl radicals.n6

2. Exogenous(Obtained from diet)

- Vitamins:Vitamins C,E,and A act as potent radical scavengers.

B. Based on Solubility

1. Water-Soluble Antioxidants

- Function in aqueous cellular compartments(cytosol,plasma).
- Examples:Vitamin C(ascorbic acid),uric acid,polyphenols.

2. Lipid-Soluble Antioxidants

- Protect lipid membranes and lipoproteins from oxidation.
- Examples:Vitamin E(α-tocopherol),carotenoids,coenzyme Q10.

C. Based on Mechanism of Action

1. Free Radical Scavengers

- Donate electrons or hydrogen atoms to stabilize free radicals.
- Examples:Flavonoids,vitamin C,vitamin E.

2. Metal Chelators

- Bind transition metals(Fe^{2+},Cu^{2+})to prevent ROS generation via Fenton reactions.
- Examples:Flavonoids,tannic acid,phytates.
- Polyphenols and Flavonoids:Found in fruits,vegetables,tea,and medicinal plants.
- Carotenoids:Beta-carotene,lutein,and lycopene; lipid-soluble pigments protecting membranes.

3. Singlet Oxygen Quenchers

- Deactivate singlet oxygen($^1O_2$),preventing oxidative damage.
- Examples:Carotenoids(β-carotene,lycopene).

4. Lipid Peroxidation Inhibitors

- Prevent oxidative degradation of polyunsaturated fatty acids in membranes.
- Examples:Vitamin E,flavonoids.

5. Synergistic Antioxidants

- Regenerate other antioxidants or enhance their activity.
- Example:Vitamin C regenerates oxidized vitamin E.

3. Plant-Derived Antioxidants

Table.2.Plant antioxidants are primarily non-enzymatic and include diverse bioactive compounds with multiple mechanisms

Class	Examples	Mechanism
Flavonoids	Quercetin,Kaempferol,Catechins	Radical scavenging,metal chelation,enzyme modulation
PhenolicAcids	Gallic acid,Caffeic acid	Free radical scavenging,lipid peroxidationInhibition

Carotenoids	Beta-carotene,Lycopene,Lutein	Singlet oxygen quenching,membraneProtection
Vitamins	C,E,A	Radical scavenging,regeneration of otherAntioxidants
Terpenoids	Eugenol,Limonene	ROS neutralization,anti-inflammatoryEffects

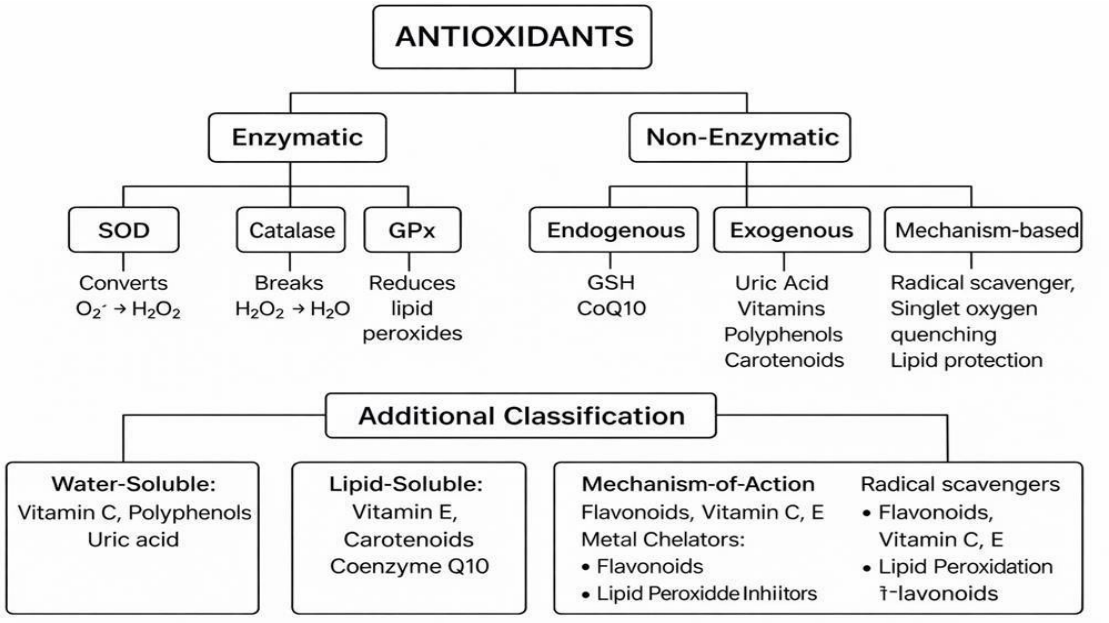

Figure 1.1.Classification of Antioxidants(Enzymatic,Non-Enzymatic,and Additional Categories)

II. Evaluation

2.1. Biological evaluation

2.1.1. DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the root extracts was evaluated using the DPPH(2,2-diphenyl-1-picrylhydrazyl)assay.A 0.1 mM DPPH solution was prepared in methanol.An aliquot of 1.0 mL of plant extract at various concentrations(20–100µg/mL)was mixed with 1.0 mL of DPPH solution.The reaction mixture was shaken well and incubated in the dark at room temperature for 30

minutes.After incubation,the absorbance was measured at 517 nm using a UV–Visible spectrophotometer against a methanol blank.Ascorbic acid was used as the standard reference compound.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\%\text{Inhibition} = (A0-A1)\times 100A0$$

whereA0 is the absorbance of the control A1 is the absorbance of the sample.Dose–response curves were plotted,and IC_{50}values were determined.

2.1.2. ABTS Radical Cation Decolorization Assay

The ABTS radical scavenging activity of the plant root extracts was determined using the $ABTS^{+}$radical cation decolorization method.The $ABTS^{+}$radical was generated by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate solution in equal volumes,and the reaction mixture was incubated in the dark at room temperature(25±2°C)for 12–16 hours to allow complete formation of the radical cation.Prior to use,the $ABTS^{+}$solution was diluted with methanol to obtain an absorbance of 0.70±0.02 at 734 nm using a UV–Visible spectrophotometer.For the assay,0.2 mL of plant extract at different concentrations(20–100µg/mL)was mixed with 2.0 mL of the diluted $ABTS^{+}$solution and incubated at room temperature for 6 minutes.After incubation,the decrease in absorbance was measured at 734 nm against methanol as blank.Ascorbic acid was used as the reference standard under the same experimental conditions.All measurements were performed in triplicate.

Calculation of ABTS Radical Scavenging Activity:The percentage inhibition of ABTS radicals by the plant extracts was calculated using the following formula:

%ABTS Radical Scavenging Activity=(A0−A1)×100A0where:A0=Absorbance of the control($ABTS^{+}$solution without sample)A1=Absorbance of the test sample or standard

The antioxidant activity of the extracts was expressed as percentage inhibition,and the IC_{50}value(concentration required to scavenge 50%of ABTS radicals)was determined from the plot of percentage inhibition versus concentration.

2.2. Data Analysis

All experimental analyses were performed in triplicate,and the results were expressed as mean±standard deviation(SD).The absorbance values obtained from antioxidant assays were first converted into percentage inhibition using the respective standard equations.Dose–response curves were generated by plotting percentage inhibition against extract concentration for each antioxidant assay.The IC_{50}values,defined as the concentration of extract required to inhibit 50%of free radicals,were calculated using linear regression analysis with a sigmoidal dose–response(variable slope)model.

2.3. Statistical InterpretationThe experimental data obtained from the phytochemical screening and antioxidant activity assays were

subjected to appropriate statistical analysis to compare the results among the root extracts of Momordica charantia,Ocimum sanctum,and Moringa oleifera.Variations in total phenolic content,total flavonoid content,and antioxidant activity were statistically evaluated to determine significant differences among the selected plant extracts.The strength of the relationship between total phenolic content,total flavonoid content,and antioxidant potential was assessed using correlation analysis,and the results were interpreted to understand the contribution of phytoconstituents to antioxidant activity.The findings were further compared with previously reported studies to validate the observed trends and to establish the scientific relevance and reliability of the present investigation.

III. RESULT & DISCUSSION

3.1. Antioxidant Activity

3.1.1. DPPH Free Radical Scavenging Assay

All extracts exhibited concentration-dependent DPPH radical scavenging activity.The percentage inhibition and IC_{50}values are shown in Table 1.3.

Table 3:DPPH Radical Scavenging Activity and IC_{50}Values

Plant Extract	IC_{50}(µg/mL)
Momordica charantia	72.4±2.6
Ocimum sanctum	58.9±2.1
Moringa oleifera	41.3±1.8
Ascorbic acid	18.6±0.9

Lower IC_{50}values indicate stronger antioxidant activity.Moringa oleifera demonstrated the highest DPPH scavenging potential among the extracts.

Table 4:DPPH Radical Scavenging Activity(%inhibition,Mean±SD)

Concentration(µg/mL)	MomordicaCharantia	Ocimumsanctum	MoringaOleifera	AscorbicAcid
20	23.8±1.1	31.6±1.3	40.9±1.5	57.4±1.2
40	36.9±1.5	47.8±1.7	58.6±1.9	72.8±1.4
60	50.7±1.8	62.9±2.0	73.2±2.2	87.6±1.6
80	62.8±2.1	75.4±2.3	86.1±2.5	95.1±1.4

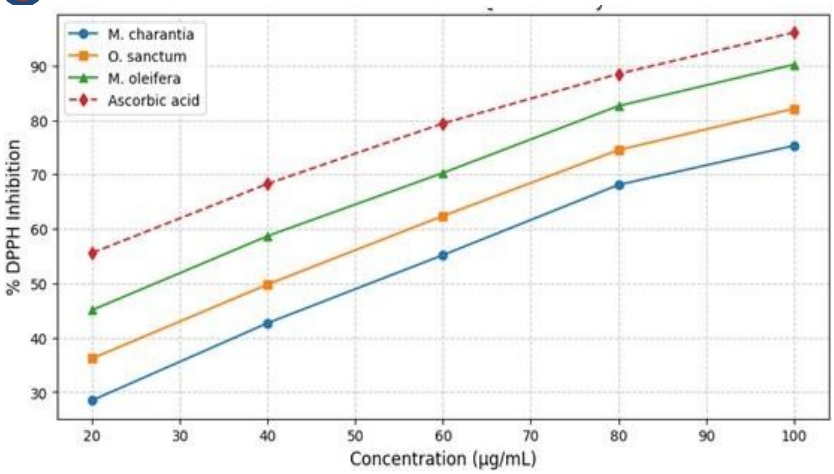

Figure 1.2:Dose–response curve of DPPH radical scavenging activity.

The DPPH radical scavenging activity of the hydroethanolic root extracts is summarized in Table 1.3 and depicted in Figure 1.2.All extracts exhibited concentration-dependent scavenging activity.Among the tested samples,Moringa oleifera demonstrated the highest percentage inhibition at all concentrations,followed by Ocimum sanctum and Momordica charantia.

The IC_{50}values(Table 1.4)further confirmed this trend,with Moringa oleifera showing the lowest IC_{50}value,indicating the strongest antioxidant activity.The superior performance of Moringa oleifera may be attributed to its higher phenolic and flavonoid contents,which enhance its ability to donate hydrogen atoms to stabilize DPPH radicals.

3.1.2. ABTS Radical Scavenging Assay

Similar trends were observed in the ABTS assay,confirming the antioxidant efficiency of the extracts.

The ABTS radical scavenging activity results are presented in Table 1.6 and Figure1.3.Similar to the DPPH assay,all extracts showed a dose-dependent increase in ABTS radical inhibition.Moringa oleifera exhibited the highest scavenging activity,followed by Ocimum sanctum and Momordica charantia.The IC_{50}values obtained from the ABTS assay(Table 1.5.)corroborated the DPPH findings.The ABTS assay is particularly useful in evaluating both hydrophilic and lipophilic antioxidant capacity,suggesting that the root extracts possess a broad spectrum of antioxidant constituents.

Table 5.ABTS Radical Scavenging IC_{50}Values

Plant Extract	IC_{50}(µg/mL)
Momordica charantia	68.1±2.3
Ocimum sanctum	54.7±1.9
Moringa oleifera	39.5±1.6
Ascorbic acid	16.9±0.7

4.5.2 ABTS Radical Cation Scavenging Assay

Table 6.ABTS radical scavenging activity(%inhibition,mean±SD)

Concentration(µg/mL)	M.charantia	O.sanctum	M.oleifera	Ascorbic acid
20	26.4±1.1	33.7±1.3	41.8 ±1.5	54.6±1.2
40	39.6±1.5	48.9±1.7	59.7 ±1.9	71.2±1.4
60	51.8±1.8	62.4±2.0	73.6 ±2.2	85.3±1.6
80	63.7±2.1	74.9±2.3	84.8 ±2.5	94.1±1.5
100	71.9±2.4	82.6±2.6	91.2 ±2.8	97.3±1.3

Figure 1.3.Dose–response curve of ABTS radical scavenging activity.

The ABTS assay confirmed the antioxidant trends observed in the DPPH assay.The ability of extracts to neutralize both hydrophilic and lipophilic radicals underscores their broad-spectrum antioxidant capacity.

IV. CORRELATION ANALYSIS DATA USED FOR CORRELATION

Table.7.Calculated Mean DPPH(%inhibition)

Plant Extract	TPC(mg GAE/g)	Mean DPPH(%inhibition)
Momordica charantia	62.4	43.6
Ocimum sanctum	78.9	54.4
Moringa oleifera	91.6	64.7

Table 8.Correlation between TPC and DPPH

s.no	TPC(mgGAE/g)	Mean DPPH(%inhibition)
TPC(mg GAE/g)	1	
Mean DPPH(%inhibition)	0.92	1

Table.9.Calculated Mean ABTS(%inhibition)

Plant Extract	TFC(mg QE/g)	ABTS%Inhibition(100µg/mL)
M.charantia	38.7	71.9
O.sanctum	52.3	82.6
M.oleifera	67.8	91.2

Table 10.Correlation between TFC and ABTS

s.no	TFC(mgQE/g)	ABTS%Inhibition(100µg/mL)
TFC(mg QE/g)	1	
ABTS%Inhibition(100µg/mL)	0.89	1

Total flavonoid content also demonstrated a strong positive correlation with antioxidant activity.The correlation coefficient between TFC and DPPH radical scavenging activity was r=0.90,while the correlation between TFC and ABTS radical scavenging activity was r=0.87.These findings indicate that flavonoids significantly contribute to the antioxidant capacity of the extracts,likely due to their ability to chelate transition metal ions,inhibit lipid peroxidation,and scavenge reactive oxygen species.

The slightly higher correlation coefficients observed with the DPPH assay compared to the ABTS assay suggest that phenolic and flavonoid compounds in the studied extracts may be more effective in scavenging stable free radicals such as DPPH.However,the strong correlations in both assays confirm that the antioxidant activity of the extracts is mediated by a broad spectrum of hydrophilic and lipophilic antioxidant constituents.

Among the tested extracts,Moringa oleifera consistently showed the highest total phenolic and flavonoid contents,which corresponded with its superior antioxidant activity in both assays.Ocimum sanctum exhibited moderate phytochemical levels and antioxidant potential,while Momordica charantia showed comparatively lower values.This consistent trend further supports the strong positive association between phytochemical concentration and antioxidant efficacy.

Table 11:Pearson's Correlation Coefficients Between Phytochemical Content andAntioxidant Activity

Total Phenolic Content(TPC)	DPPH	0.92	Very strong Positive

PhytochemicalParameter	AntioxidantAssay	CorrelationCoefficient(r)	Strength of Correlation
Total Phenolic Content(TPC)	ABTS	0.89	Strong positive
Total Flavonoid Content(TFC)	DPPH	0.90	Very strong positive

Total Flavonoid Content(TFC)	ABTS	0.87	Strong positive

Pearson's correlation coefficient(r)values closer to+1 indicate a stronger positive relationship between phytochemical content and antioxidant activity.All correlations were statistically significant at $p<0.05$.

Figure 1.4:Correlation plot between TPC and DPPH scavenging activity.

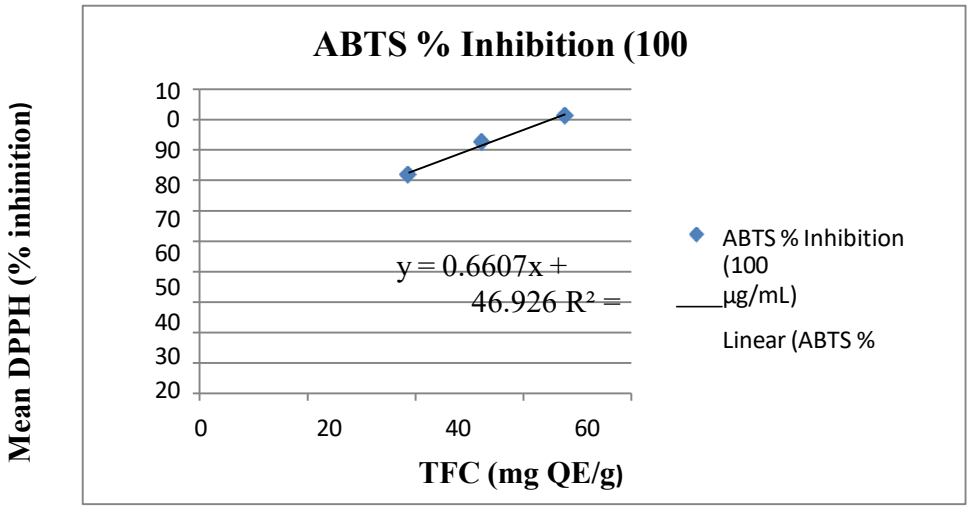

Figure 1.5.:Correlation plot between TFC and ABTS scavenging activity.

V. CONCLUSION

The present study demonstrated the comparative phytochemical composition and antioxidant potential of hydroethanolic root extracts of Momordica charantia,Ocimum sanctum,and Moringa oleifera.All extracts were found to contain important bioactive compounds such as phenolics,flavonoids,tannins,saponins,and alkaloids,which contribute to antioxidant activity.The hydroethanolic solvent system proved effective in extracting a wide range of phytoconstituents.Among the selected plants,Moringa oleifera exhibited the highest extractive yield,indicating a richer phytochemical profile.It also showed the highest total phenolic and flavonoid contents,which are key contributors to antioxidant activity.The antioxidant assays(DPPH and ABTS)revealed a concentration-dependent increase in radical scavenging activity for all extracts.Moringa oleifera demonstrated the lowest IC_{50}values,indicating the strongest antioxidant potential.Ocimum sanctum showed moderate activity,while Momordica charantia exhibited comparatively lower antioxidant capacity.A strong positive correlation was observed between phytochemical content and antioxidant activity.These findings confirm that phenolics and flavonoids play a major role in free radical scavenging.The study supports the traditional

medicinal use of these plants in managing oxidative stress.Overall,Moringa oleifera emerged as the most potent natural antioxidant source among the studied extracts.

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