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GC-MS Analysis and In-Vitro Antioxidant Activity of Ethanolic Flower Extracts of *Hibiscus Sabdariffa Linn* and *Moringa Oleifera* from Bhagalpur Region

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Abstract: ***The escalating global incidence of oxidative stress-related pathologies has intensified the scientific pursuit of plant-derived therapeutics. This investigation evaluates the in vitro antioxidant efficacy of Hibiscus sabdariffa Linn and Moringa oleifera flowers, two phytochemically rich medicinal plants, with a focus on elucidating their bioactive constituents via Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Antioxidant capacities were quantified through DPPH, phosphomolybdate, Hydrogen peroxide and Ferric reducing power assays. The results revealed that both plant extracts exhibited robust antioxidant activity and their scavenging activities are in the order of H_2O_2>DPPH>Phosphomolybdate>FRAP. The chemical profiling of ethanolic flower extracts of Kudrum (Hibiscus sabdariffa Linn.) and drumstick tree (Moringa oleifera) using gas chromatography-mass spectrometry (GC-MS), identified a total of 45 compounds, with 24 from H. sabdariffa Linn. and 21 from M. oleifera. The ethanolic extract of H. sabdariffa Linn. flowers exhibited a high number of prominent peaks with key chemical constituents such as n-hexadecanoic acid, beta-Amyrin acetate, Ursolic aldehyde, .beta.-Amyrin, Tetradecanoic acid and Villosin. In contrast, the major components identified in M. oleifera flowers included Trimyristin, 4-oxide, (R)-, 1,1-diethoxyethane, Ethyl Oleate, Hexadecanoic acid and Methyl 9,10-octadecadienoate. These findings underscore the potential of Hibiscus sabdariffa Linn. and Moringa oleifera flowers as promising sources of natural antioxidants.***

Keywords: GC-MS, *Hibiscus sabdariffa Linn., Moringa oleifera*, antioxidant, DPPH

1. Introduction

The global rise in oxidative stress-related diseases has prompted the exploration of novel therapeutic strategies to mitigate their health and economic burdens. Conventional pharmacological treatments, while effective, are often associated with adverse side effects and high costs [1]. This has led to a growing interest in plant-based remedies, particularly those rich in bioactive phytochemicals with antioxidant properties. Medicinal plants have long been a cornerstone of traditional medicine due to their rich repository of bioactive compounds with diverse therapeutic properties. Among these, *Hibiscus sabdariffa Linn.* (roselle or kudrum) and *Moringa oleifera* (drumstick tree) have garnered significant attention for their potential health benefits. Both plants are widely utilized in various cultures for their nutritional, medicinal, and therapeutic applications [2,3]. The flowers of *H. sabdariffa Linn.* and *M. oleifera* are two edible flowers found commonly in Bihar and Jharkhand region. *M. oleifera*, commonly referred to as the "miracle tree," is known for its wide range of therapeutic properties, including antioxidant, anti-inflammatory, and antidiabetic effects [4]. These bioactivities are attributed to its rich content of flavonoids, phenolic acids, glucosinolates, and isothiocyanates, which have demonstrated significant potential in the modulation of oxidative stress [5]. *H. sabdariffa Linn.*, widely known as roselle, is similarly valued for its pharmacological potential, particularly its antioxidant and antidiabetic activities. The presence of anthocyanins, flavonoids, and phenolic acids in Hibiscus sabdariffa underpins its ability to scavenge free radicals [6].

Antioxidants play a critical role in neutralizing free radicals and reactive oxygen species (ROS), thereby mitigating oxidative stress, which is implicated in the pathogenesis of numerous chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. Plant extracts rich in bioactive phytochemicals, such as polyphenols (gallic acid), flavonoids (quercetin), and terpenoids (ursolic acid), have demonstrated the ability to neutralize free radicals and mitigate conditions associated with oxidative stress [7]. In-vitro antioxidant assays provide valuable insights into the radical-scavenging potential of plant-derived extracts, highlighting their therapeutic promise. Gas chromatography-mass spectrometry (GC-MS) is a powerful analytical technique for profiling the chemical constituents of complex plant extracts. The identification of key bioactive compounds through GC-MS not only aids in understanding the chemical composition of plant extracts but also establishes a basis for correlating these compounds with biological activities.

The present study aims to investigate the chemical composition and in-vitro antioxidant activity of *H. sabdariffa* and *M. oleifera* flower extracts sourced from the Bhagalpur region. By employing GC-MS analysis and antioxidant assays, this research seeks to elucidate the phytochemical profiles and evaluate the radical-scavenging efficacy of these locally available plants. The findings will contribute to the growing body of knowledge on the medicinal properties of *H. sabdariffa* and *M. oleifera* and their potential applications in health and wellness.

2. Material and Methods

Chemicals and Reagents:
All the chemicals used in the present study are of analytical grade bought from Loba Chemicals. The distilled water used is x-tra pure bought from sigma chemicals and PDA used is bought from Readymed. Aluminium chloride used in estimation of total phenolic content is bought from Merck.

Preparation of Plant Extract:
The flowers of *H. sabdariffa Linn.* and *M. oleifera* were bought from local market of Bhagalpur, Bihar, India. The flowers were washed with running water 7-10 times and then two times with distilled water. After complete draining of water, the flowers were kept in fridge for three weeks. After drying, the flowers were powdered separately in Usha Colt Mixer jar not letting the jar heat. The extract of *H. sabdariffa Linn* and *M. oleifera* flowers was prepared by maceration technique [8]. The powdered flowers were separately soaked in pure ethanol for 48 hours, then filtered through normal filter paper. The filtrate was centrifuged and again filtered through Whatman's filter paper 41. The filtrate thus obtained was dried over water bath. The obtained semi solid is dry ethanolic extract. Hence two ethanolic extracts were prepared, *H. sabdariffa Linn.* flower extract (KF) and *M. oleifera* flower extract (MOF).

Antioxidant Activity Assays:
Antioxidant activity of ethanolic extract of *H. sabdariffa Linn* and *M. oleifera* flowers was assessed by Ferric Reducing Power assay [9], DPPH [10], H_2O_2 scavenging Assay [11] and Phosphomolybdate assay [12]. Each extract was dissolved in absolute ethanol to prepare the stock solution of concentration of 1mg/ml.

Ferric Reducing Power Assay:
Ethanolic extract samples of various concentrations were taken and the volume was raised to 1ml by adding respective solvent. Then the phosphate buffer (0.2M and pH 6.6) was added followed by addition of 1% Potassium ferricyanide. The solution was incubated at 50°C for 20 min. The 10% trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The supernatant was diluted with deionized water followed by addition of 0.1% ferric chloride solution. The absorbance was recorded at 700 nm. The BHT was taken as standard. The results are expressed as BHT equivalent per ml of plant part extract.

Phosphomolybdate assay:
Phosphomolybdate reagent was prepared by adding equal parts of 0.6M sulfuric acid, 28mM sodium phosphate and 4 mM ammonium molybdate. This reagent was added to different concentrations of ethanolic plant extracts and the solution was incubated at 90°C for 90 min and then cooled at room temperature. The absorbance was taken at 695nm. BHT was used as standard and the results are expressed as BHT equivalent per mg of dry plant extract. Every experiment was performed three times and mean of absorbances are calculated and expressed as mean ± standard deviation.

DPPH Assay:
The solution of 10^{-4} dilution of DPPH(2,2-diphenyl-1-picrylhydrazyl) was prepared in pure ethanol. 1ml of this solution was added to 2ml of plant extract solution in ethanol of different dilutions. The mix was incubated at room temperature for 15 min and then the absorbance was noted at 517 nm. The control is the solution of DPPH mixed with equal volume of solvent without plant extract. The % DPPH inhibition activity was calculated as follows:

$$\% \text{ DPPH Inhibition Activity} = (A_C - A_S) / A_C \times 100$$

Where, A_C = absorbance of control
A_S = Absorbance of sample
Hydrogen Peroxide Scavenging Assay:

The 40mM solution of Hydrogen peroxide was prepared in phosphate buffer of pH 7.4. 0.6ml of this solution was added to different dilutions of plant extract solution. The mix was allowed to rest for 10 min and then the absorbance was noted at 230 nm. The control was equal volume of hydrogen peroxide added to solvent used. The % H_2O_2 Scavenging activity was calculated as follows:

$$\% H_2O_2 \text{ Scavenging activity} = (A - a) / A \times 100$$

Where, "A" is absorbance of control and "a" is Absorbance of sample

Total Phenolic Content:
Total phenolic content was estimated by using Folin-Cio-Calteau Reagent [13]. Briefly, to an aliquot of 500µl of extract in ethanol, 500µl of Folin-cio-calteau reagent is added then the solution is diluted by adding ml of deionized distilled water followed by addition of 1.5 ml of 20% sodium carbonate solution and again 1.9 ml of deionized distilled water was added. After incubation for 2 hours, the absorbance was recorded at 760nm using Systronics – 117- UV – VIS Spectrophotometer. Gallic acid served as the reference phenolic compound, and the results were reported as Gallic Acid Equivalents (GAE) per gram of extract.

Total Flavonoid content:
Total flavonoid content was estimated by aluminium chloride method [14]. To an aliquot of 500µl of extract, 4ml of ethanol and 1ml of 10% aluminium chloride was added followed by addition of 1ml of 1M sodium acetate solution. The absorbance was noted after incubation for 45min in dark at 420 nm using Systronics 117 UV-VIS Spectrophotometer. Quercetin was used as standard and the results were expressed as Quercetin equivalent (QE) per gram of extract.

Phytochemical Screening:
The phytochemicals present in the ethanolic extract of *Hibiscus sabdariffa Linn* and *Moringa oliefera* flowers were detected by using qualitative analysis for different phytochemicals like alkaloids, glycosides, carbohydrates, flavonoids etc. [15]

GC-MS Analysis:
The GC-MS analysis was conducted using an Agilent 7890B GC system coupled with an Agilent 5977A MSD, utilizing an HP-5MS capillary column (30 m x 0.25 mm x 0.25 µm). The helium was used as the carrier gas at a flow rate of 1 mL/min. Samples were injected in split mode with a 10:1 split ratio. The oven temperature program started at 50°C with a hold for

2 minutes, followed by an increase at 10°C per minute to 150°C, where it was hold for 2 minutes. The temperature then increased at 5°C per minute to 250°C, with another 2-minute hold, and finally, it was ramped at 10°C per minute to 280°C, holding for 5 minutes. The injector and transfer line temperatures were set at 250°C and 100°C, respectively. The mass spectrometry conditions included an ion source temperature of 230°C, a quadrupole temperature of 150°C, a mass range of 35-500 m/z, and a solvent delay of 3 minutes, with a scan rate of 1.562 scans per second. The NIST14.L library (2020) was subsequently searched to compare the compound structures with those in the NIST database. Compounds were then identified based on the retention times and mass spectra with already known compounds in the NIST library (C:\Database\NIST20.1)

Statistical Analysis:
The experiments for antioxidant activity were done in triplicates. The values are calculated as mean with standard deviation using Microsoft excel. TFC and TPC were analyzed twice and values are reported as mean with standard deviation. The significance of results was analyzed using ANOVA: single factor($p<0.05$).

3. Results

Phytochemical Screening:
The qualitative analysis of ethanolic extract of *Hibiscus sabdariffa Linn* and *Moringa oliefera* flowers shows the presence of various phytochemicals as shown in Table1.

Table 1: Qualitative phytochemical Analysis

Phytochemical Component	Ethanolic extract of *Hibiscus sabdariffa Linn.* Flower (KF)	Ethanolic Extract of *Moringa oliefera* flower (MOF)
Alkaloids	-	+
Carbohydrates	+	+
Flavonoids	+	+
Coumarins	+	+
Glycosides	+	+
Phlobatannins	-	-
Terpenoids	+	+
Phenols	+	+

The ethanolic extract of flowers of *Hibiscus sabdariffa Linn* and *Moringa oleifera* shows the presence of various phytochemicals such as flavonoids, phenolics, terpenoids, coumarins, Glycosides and carbohydrates. The qualitative test for alkaloids and phlobatannins was negative for ethanolic extract of flower of *Hibiscus sabdariffa Linn* whereas the test was found to be positive for ethanolic extract of flower of *Moringa oleifera*.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC):
TPC of KF and MOF is calculated using the linear regression equation $y=0.0007x+0.0559$, with coefficient of determination R^2 =0.9676 from the calibration curve for Gallic acid and TFC is calculated using the equation $y=0.0224x+0.1422$, with coefficient of determination R^2 = 0.965 from the calibration curve for quercetin. The high value of R^2 indicates the strong linear relationship between the concentration and the absorbance recorded. It shows the reliability of standard curve used for calculating TPC and TFC. The total phenolic content (TPC) of *Moringa oleifera* flowers (MOF) is 107.429±2.02 µg GAE/mg of dry extract, which is significantly higher than that of *Hibiscus sabdariffa Linn* flowers (KF), measured at 66±8.08 µg GAE/mg of dry extract. Similarly, the total flavonoid content (TFC) of MOF is 31.356±0.94 µg QE/mg of dry extract, surpassing the TFC of KF, which stands at 19.594±0.94 µg QE/mg of dry extract. These findings indicate that *Moringa oleifera* flowers contain a higher concentration of phenolic compounds and flavonoids compared to *Hibiscus sabdariffa Linn* flowers. The numerical values as shown in the Table 2 are means of duplicate experiments along with standard deviation as calculated on MS excel. The values are also statistically significant at $P<0.05$ as determined through single-factor ANOVA.

Table 2: TFC and TPC

Ethanolic Extract of Flowers	TFC as QE µg/mg of dry extract	TPC as GAE in µg/mg of dry extract
Hibiscus Sabdariffa Linn.(KF)	19.594 ± 0.94	66 ± 8.08
Moringa oleifera (MOF)	31.356 ± 0.94	107.429 ± 2.02

Antioxidant Activity:
The antioxidant activity of KF and MOF was evaluated using four assays: Phosphomolybdate, Ferric Reducing Power, DPPH and hydrogen peroxide scavenging assays. Butylated Hydroxy Toluene (BHT) was used as standard antioxidant. The results of these assays are expressed as milligrams BHT equivalent per milligram of dry extract as in Table 3.

Table 3: mg BHT eq/mg of dry extract.

	DPPH Scavenging Assay	H_2O_2 Scavenging Assay	FRAP Assay	Phosphomolybdate Assay
Hibiscus sabdariffa Linn (KF)	1.23 ± 0.298	1.291 ± 0.089	0.107±0.139	0.612 ± 0.086
Moringa oleifera (MOF)	1.077 ± 0.145	1.187 ± 0.17	0.136±0.082	0.449 ± 0.154

The absorbance results for the phosphomolybdate and Ferric reducing power assays for KF, MOF and BHT are presented in Fig. 1(a) and (b). Among these, BHT exhibited the highest absorbance, followed by KF, while MOF showed the lowest. At a concentration of 0.6 mg/ml in the phosphomolybdate assay, the absorbance values for KF, MOF and BHT were 0.341, 0.287, and 0.487 nm, respectively. Since higher absorbance correlates with greater antioxidant power, these results demonstrate the superior antioxidant capacity of BHT

compared to KF and MOF. In this assay, antioxidants reduce Mo(VI) to Mo(V), forming a green-colored complex with absorbance at 695 nm. KF showed a greater reduction of Mo(VI) to Mo(V) than MOF, indicating a higher concentration of antioxidants in KF. When expressed as milligrams of BHT equivalent per milligram of dry extract, MOF contained 0.449 ± 0.154 mg BHT eq/mg, while KF contained 0.612±0.086 mg BHT eq/mg (Table 3). These values highlight the greater Mo(VI) reducing power of KF compared to MOF.

The ferric reducing antioxidant power (FRAP) assay was also used to assess the reducing potential of the extracts by measuring the reduction of Fe^{3+} to Fe^{2+}, which forms a Prussian blue complex with absorbance at 700 nm. From Figure 1(b), it is evident that BHT displayed higher absorbance than MOF and KF. This indicates that KF and MOF had low power to reduce Fe^{3+} to $Fe^{2+}$with respect to BHT. At a concentration of 0.3 mg/ml, the absorbance values for KF, MOF and BHT in the FRAP assay were 0.062, 0.067 and 0.183 nm, respectively. The FRAP values, expressed as milligrams of BHT equivalent per milligram of dry extract (Table 3), confirm the superior reducing power of BHT as compared to MOF and KF.

DPPH is a synthetic stable radical that is used for the determination of the free radical scavenging activity of antioxidants. In the present study, the free radical scavenging activity of antioxidants, if present, in the ethanolic extract of *Moringa oleifera* and *Hibiscus sabdariffa Linn* flowers, was determined by using DPPH and hydrogen peroxide assay taking BHT as standard. The percentage inhibition of DPPH and hydrogen peroxide is shown in Figures 2(a) and 2(b) respectively. In this assay, the percentage free radical scavenging activity of KF and MOF is almost similar and is higher than BHT. The IC50 (concentration of sample for 50% inhibition of free radical) values of KF, MOF and BHT are shown in the form of a bar chart in Figure 3. It is clear from Figure 3 that the IC50 value of BHT is highest which is 581.927 µg/mL and is lowest for KF which is 522.296 µg/mL in DPPH Assay, and for MOF is 568.84 µg/mL showing more free radical scavenging capacity of KF extract as compared to BHT and MOF extract. The IC50 values are in the same order in hydrogen peroxide assay, also the values for KF, MOF and BHT are 142.821, 150.8729 and 185.05 µg/mL, respectively. Hydrogen peroxide scavenging assay of antioxidant activity shows that higher percentage of H_2O_2 being scavenged by KF, as compared to MOF and BHT. BHT is a synthetic antioxidant which also decreases the concentration of hydrogen peroxide in the solution with its increasing concentration. In DPPH and hydrogen peroxide scavenging assays, the scavenging activity of KF and MOF extracts is higher than BHT, showing larger antioxidant activity of KF and MOF.

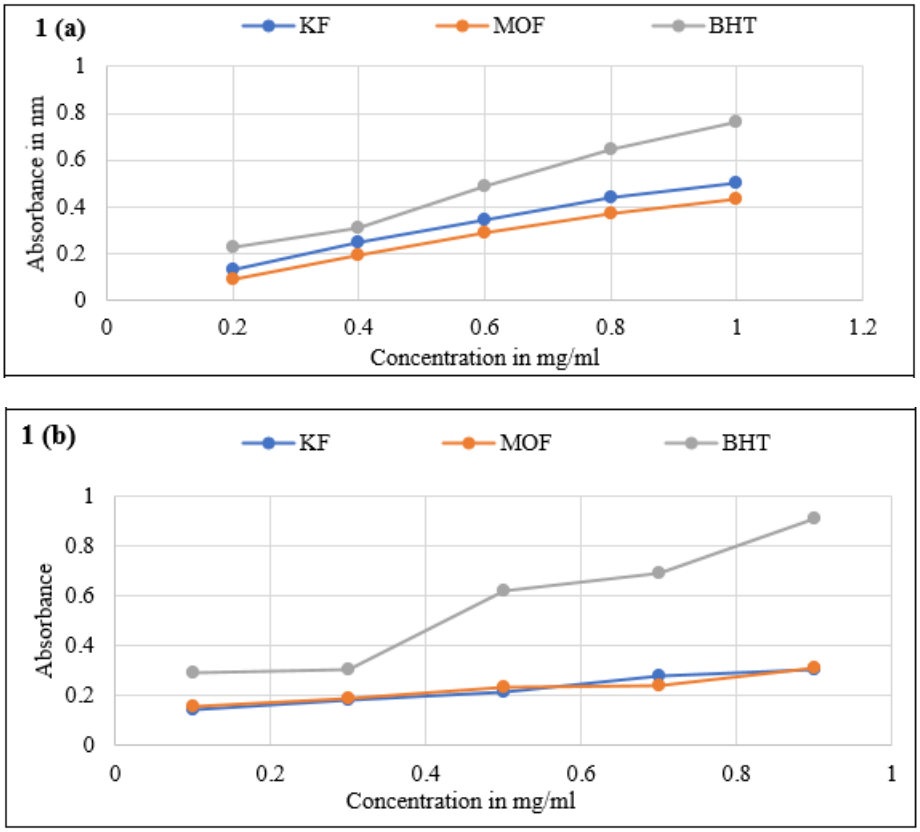
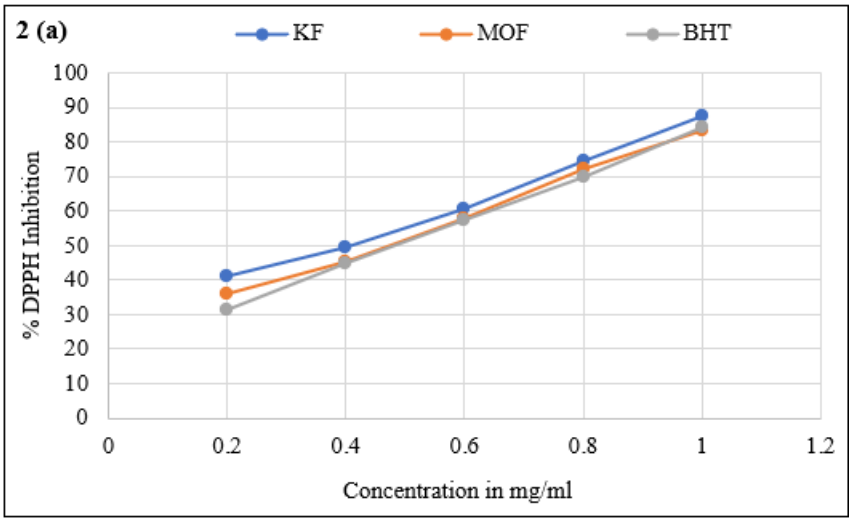


Figure 1: (a) Phosphomolybdate assay showing absorbance of *H. sabdariffa Linn.* flower extract (KF), *M. oleifera* flower extract (MOF) and Butylated Hydroxy Toluene (BHT); (b) Ferric Reducing Antioxidant Power assay showing absorbance of *H. sabdariffa Linn.* flower extract (KF), *M. oleifera* flower extract (MOF) and Butylated Hydroxy Toluene (BHT);



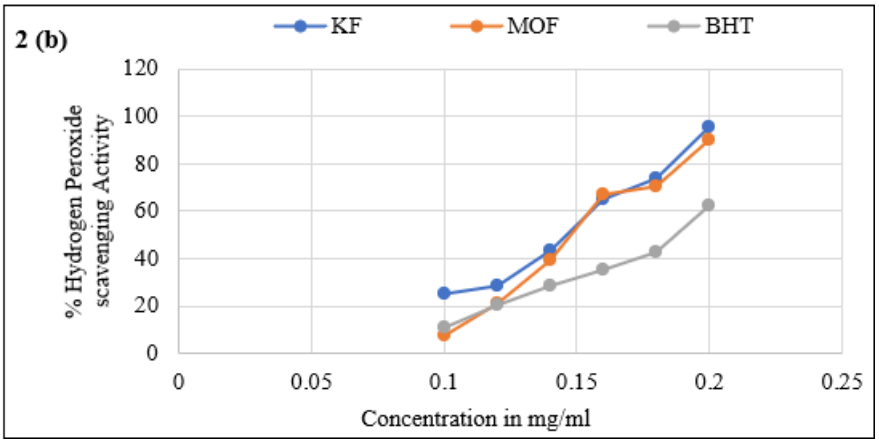


Figure 2: (a) DPPH assay showing percentage inhibition of DPPH by *H. sabdariffa Linn.* flower extract (KF), *M. oleifera* flower extract (MOF) and Butylated Hydroxy Toluene (BHT); (b) H_2O_2 Inhibition Assay showing percentage inhibition activity by *H. sabdariffa Linn.* flower extract (KF), *M. oleifera* flower extract (MOF) and Butylated Hydroxy Toluene (BHT).

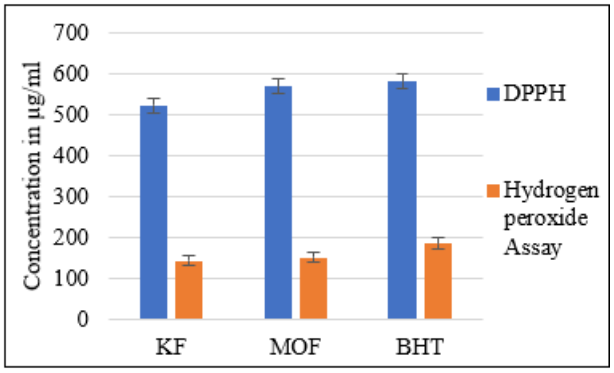


Figure 3: IC50 values in free radical scavenging Assays of *H. sabdariffa Linn.* flower extract (KF), *M. oleifera* flower extract (MOF) and Butylated Hydroxy Toluene (BHT)

GC-MS Analysis for the presence of phytochemicals

The chemical compounds identified by GC-MS analysis in the ethanolic extracts of flowers of *Hibiscus sabdariffa Linn.*(KF) and *Moringa oleifera* (MOF) are presented in Table 4 and 5. Their respective GC-MS chromatograms are shown in Figure 4 and 5. From the chromatogram of ethanolic extract of *Hibiscus sabdariffa Linn.* flowers, 24 compounds were identified of which the most abundant compounds were beta-Amyrin acetate (Olean-12-en-3-ol, acetate, (3.beta.)-), Ursolic aldehyde, beta.-Amyrin, Tetradecanoic acid, 2-hydroxy-1,3-propanediyl ester, Villosin, Hexadecanoic acid and ethyl hexadecanoate.

In the ethanolic extract of *Moringa oleifera* flowers (MOF), 21 compounds were identified by GC-MS Chromatography. The most abundant compounds are Trimyristin, n-Hexadecanoic acid, 1,1-diethoxyethane, Methyl 9,10-octadecadienoate, Ethyl Oleate, Hexadecanoic acid, ethyl ester.

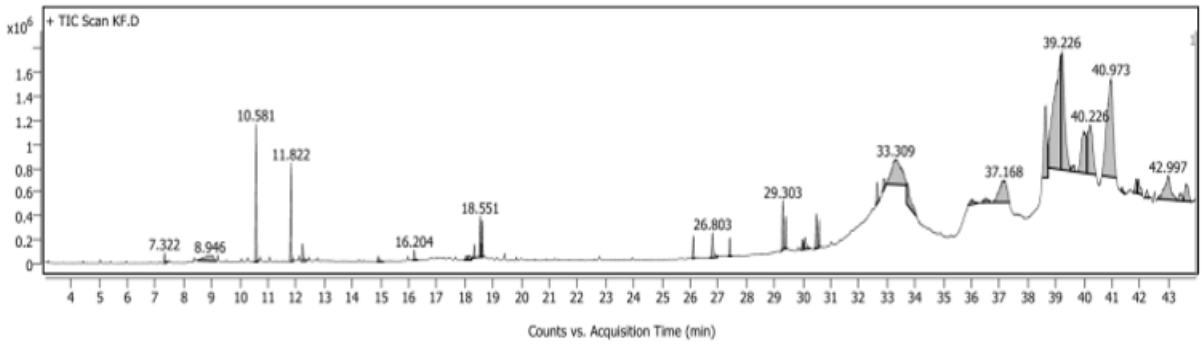


Figure 4: GC-MS Chromatogram of ethanolic extract of *Hibiscus sabdariffa Linn. Linn.* Flowers (KF)

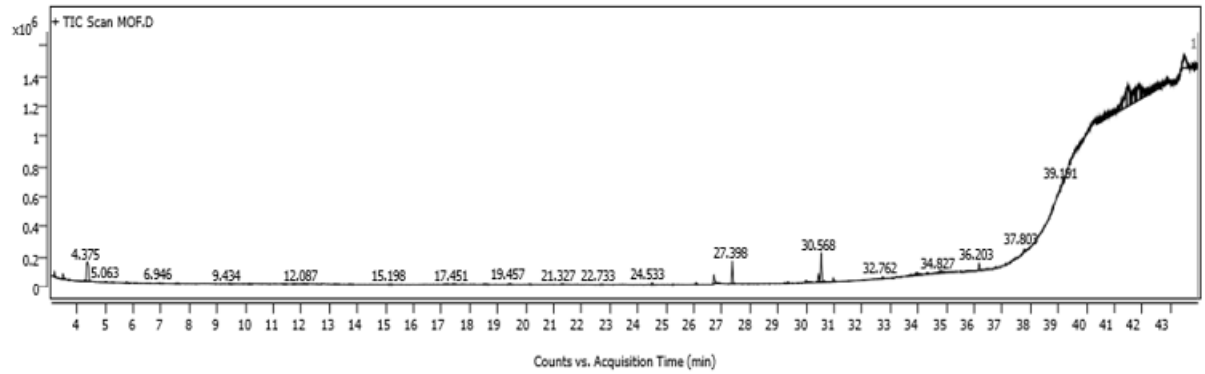


Figure 5: GC-MS Chromatogram of ethanolic extract of *Moringa oleifera* Flowers (MOF)

Table 4: Compounds identified in the ethanolic extract of *Hibiscus sabdariffa Linn.* Flowers (KF)

S. No.	Compound Name	Formula	RT(min)	Area %
1	1H-Tetrazole, 1-methyl-	$C_2H_4N_4$	7.322	0.22
2	Propylamine, N, N,2,2-tetramethyl-, N-oxide	$C_7H_{17}NO$	8.946	1.29
3	5-Amino-3H- [1,2,3] triazole-4-carboxylic acid, hydrazide	$C_3H_6N_6O$	10.581	2.48
4	4-Ethylcyclohexyl ethylphosphonofluoridate (isomer 1)	$C_{10}H_{20}FO_2P$	11.822	1.83
5	Octanedioic acid, dimethyl ester	$C_{10}H_{18}O_4$	16.204	0.24
6	cis-Calamenene	$C_{15}H_{22}$	18.163	0.29
7	Nonanedioic acid, dimethyl ester	$C_{11}H_{20}O_4$	18.339	0.31
8	Quinoline, 4-methyl-, 1- oxide	$C_{10}H_9NO$	18.551	1.16
9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	26.115	0.53
10	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	26.803	1.00
11	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	27.415	0.46
12	Methyl 9,10- octadecadienoate	$C_{19}H_{34}O_2$	29.303	1.20
13	.beta.-D- Mannofuranoside, 2,3:5,6-di-ethylboranediyl-cis- Nerolidyl	$C_{25}H_{42}B_2O_6$	29.403	0.78
14	D-Norandrostan-16-ol, acetate, (5.alpha.,16.beta.)-	$C_{20}H_{32}O_2$	32.915	0.44
15	.beta.-Amyrin	$C_{30}H_{50}O$	33.309	6.07
16	.alpha.-Amyrin	$C_{30}H_{50}O$	35.991	0.45
17	13-Hydroxy-7,14- labdadiene-6-one	$C_{20}H_{32}O_2$	36.532	0.43
18	Villosin	$C_{20}H_{28}O_2$	38.644	5.38
19	Olean-12-en-3-ol, acetate, (3.beta.)-	$C_{32}H_{52}O_2$	39.179	19.61
20	1-[3-(2,6,6-Trimethyl-cyclohex-2-enyl)-4,5- dihydro-3H-pyrazol-4-yl]-ethanone	$C_{14}H_{22}N_2O$	39.997	5.24
21	Tetradecanoic acid, 2-hydroxy-1,propanediyl Ester	$C_{31}H_{60}O_5$	40.226	6.06
22	Ursolic aldehyde	$C_{30}H_{48}O_2$	40.973	15.00
23	Lupeol	$C_{30}H_{50}O$	41.373	0.20
24	Epilupeol; 20(29)-Lupen- 3alpha-ol (isomer 2)	$C_{30}H_{50}O$	41.867	0.69

Table 5: Compounds identified in the ethanolic extract of *Moringa oleifera* Flowers (MOF)

S. No.	Name of the Compound	Formula	RT (min)	Area %
1	Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$	4.375	6.37
2	Diazene, bis(3- methylcyclohexyl)-, 1,2- dioxide	$C_{14}H_{26}N_2O_2$	6.946	0.08
3	Diphosphoric acid, diisooctyl ester	$C_{16}H_{36}O_7P_2$	9.434	0.22
4	3-Methyl-4-(phenylthio)- 2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	$C_{14}H_{16}O_2S_2$	21.327	0.33
5	3-Cyclohexylthiolane,5,5- dioxide	$C_{10}H_{18}O_2S$	12.087	0.30
6	4-Ethyl-1-hexyn-3-ol	$C_8H_{14}O$	15.198	0.16
7	7,10,10-Trimethyl-4-oxa 3,5diazatricyclo[5.2.1.0(2,6] deca-2,5-dien-3-one	$C_{10}H_{14}N_2O_2$	17.451	0.19
8	1-Octyn-3-ol, 4-ethyl-	$C_{10}H_{18}O$	18.533	0.27
9	Adamantane, 1-isothiocyanato-3-methyl-	$C_{12}H_{17}NS$	19.457	0.26

10	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	22.733	0.10
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	26.745	1.77
12	10-Hydroxydecanoic acid	$C_{10}H_{20}O_3$	26.903	0.10
13	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	27.398	3.55
14	Undec-10-ynoic acid, 3-methylbut-2- en-1-yl ester	$C_{16}H_{26}O_2$	29.386	0.31
15	Cyclohexanecarboxamide, N- furfuryl-	$C_{12}H_{17}NO_2$	30.027	0.90
16	Methyl 9,10-octadecadienoate	$C_{19}H_{34}O_2$	30.468	1.57
17	Ethyl Oleate	$C_{20}H_{38}O_2$	30.568	5.68
18	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	31.003	0.63
19	Oxacyclotetradecan-2-one, 14- methyl-	$C_{14}H_{26}O_2$	32.762	0.36
20	Trihexadecyl borate	$C_{48}H_{99}BO_3$	36.203	1.42
21	Trimyristin	$C_{45}H_{86}O_6$	41.462	6.96

4. Discussion

Ethanol is a polar solvent capable of extracting a broad spectrum of phytochemicals, including both polar and moderately non-polar compounds. Its efficacy in detecting diverse phytochemicals in plants demonstrates its suitability for isolating bioactive compounds from plant materials. Qualitative phytochemical screening of ethanolic extracts from *Hibiscus sabdariffa Linn.* and *Moringa oleifera* flowers revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids, consistent with findings by previous researchers [16, 17]. These bioactive compounds contribute to the antioxidant, antimicrobial, anti-inflammatory, and therapeutic properties of the plants, supporting their traditional medicinal uses [18]. The ethanolic extract of *Moringa oleifera* and *Hibiscus sabdariffa Linn* flowers exhibited significant phenolic and flavonoid content, reflecting their antioxidant potential. The total phenolic content (TPC) of the ethanolic extract of *Moringa oleifera* flowers in this study was determined to be 107.429 mg/g of gallic acid equivalent, which is significantly higher than the 19.31 mg/g reported by Alhakmani et al. [19]. Meanwhile, the TPC of the ethanolic extract of *Hibiscus sabdariffa* Linn was found to be 66 mg/g of gallic acid equivalent, closely matching the 70.38 mg/g reported by Lade et al. [20] and slightly exceeding the 41.07 mg/g reported by Sirag et al. [21].

The antioxidant activity of ethanolic extracts from *Hibiscus sabdariffa Linn* and *Moringa oleifera* flowers was evaluated through various In Vitro assays, including DPPH radical scavenging, hydrogen peroxide scavenging, ferric reducing antioxidant power and the phosphomolybdate assay. Using multiple methods provides a comprehensive evaluation of biological activity [22]. The DPPH assay revealed strong free radical scavenging capacity in both extracts, aligning with research emphasizing natural antioxidants as alternatives for managing oxidative stress [23]. The results indicated concentration-dependent inhibition of free radicals by both extracts. Additionally, both extracts effectively scavenged hydrogen peroxide, a reactive oxygen species associated with oxidative stress and cellular damage. This activity is likely attributed to phenolics and flavonoids, which donate electrons to convert hydrogen peroxide into water. The reducing power assay, which measures the ability of compounds to reduce ferric ions to ferrous ions, highlighted the dose-dependent reducing capacity of both extracts. In the phosphomolybdate assay, both extracts exhibited significant total antioxidant capacity, demonstrating their ability to reduce Mo (VI) to Mo(V). This assay underscores the cumulative antioxidant effects of all components in the extracts, validating their health-promoting potential. Notably, the ethanolic extract of *Hibiscus sabdariffa Linn* flowers demonstrated superior reducing power compared to the *Moringa oleifera* extract, while both showed comparable free radical scavenging activity in the DPPH and H_2O_2 assays. These findings validate the traditional medicinal applications of these plants and highlight their potential in managing oxidative stress-related disorders. Previous studies, such as those by Olasehinde et al. and Abdulkadir et al., corroborate the antioxidant efficacy of *Moringa oleifera* flowers, consistent with the current study [24, 25]. However, the findings for *Hibiscus sabdariffa Linn* do not fully align with Shafirany et al. [26], who reported an IC_{50} value of 63.77 µg/mL for the DPPH assay, compared to 522.296 µg/mL in this study. Variations may stem from biological and environmental factors, such as climatic conditions, collection location, and timing. The extracts of both plants exhibited scavenging activities in the order of H_2O_2>DPPH>Phosphomolybdate>FRAP. In vitro antioxidant activity of ethanolic flower extract of flowers of *Hibiscus sabdariffa Linn* and *Moringa oleifera* were also reported by other researchers [27, 28].

The variability in the biological activities of plant extracts can be attributed to their phytoconstituents. GC-MS analysis of the extracts revealed the presence of 24 compounds in the ethanolic extract of *Hibiscus sabdariffa Linn* (KF) and 21 compounds in the extract of *Moringa oleifera* flowers (MOF). Both extracts contained Hexadecanoic acid and its ethyl ester. Hexadecanoic acid, also known as palmitic acid, is a saturated fatty acid that has also been previously detected in the flower extract of *Hibiscus sabdariffa Linn* as reported in literature [29]. Plant extracts containing hexadecanoic acid, such as those examined in this study, have demonstrated antioxidant activity [30]. Additionally, hexadecanoic acid has been reported to possess antibacterial, antioxidant, hypocholesterolemic, anti-androgenic, haemolytic, and 5-alpha-reductase inhibitory properties [31, 32]. Methyl palmitate, detected in *Moringa oleifera*, functions as a metabolite and belongs to the category of long-chain fatty acid esters [33]. Ethyl hexadecanoate is formed through the formal condensation of the carboxyl group of palmitic acid with the hydroxyl group of ethanol. This compound functions as a plant metabolite and belongs to the categories of hexadecanoate esters and long-chain fatty acid ethyl esters [34].

Terpenoids such as ursolic aldehyde, lupeol, epilupeol, 13-hydroxy-7,14-labdadiene-6-one, α-amyrin, and cis-calamenene were detected in the GC-MS analysis of the ethanolic flower extract of *Hibiscus sabdariffa Linn.* The presence of terpenoids in this extract has also been reported

by other researchers [35]. Additionally, alpha and beta amyrin were also identified in the seed extract of *Hibiscus sabdariffa Linn.* [36]. Alpha amyrin is known to inhibit the growth of *Streptococcus* in the oral cavity [37]. The essential oils having cis-calamenene as one of its compositions have been reported to have antioxidant, antimicrobial and hemolytic properties [38].

The ethanolic extract of *Moringa oleifera* flowers revealed the presence of linoleic acid derivatives, which are known for their antioxidant and antimicrobial properties [39, 40]. Trimyristin, detected in the extract, has similar activities and is also found in nutmeg [41]. Undec-10-ynoic acid, 3-methylbut-2-en-1-yl ester, another bioactive compound, was identified in the GC-MS analysis of the ethanolic extract of *Moringa oleifera* flowers. Studies by other researchers have shown that esters of undec-10-ynoic acid possess antioxidant activity [42].

The ethanolic extracts of flowers of both plants *Hibiscus sabdariffa Linn* and *Moringa oleifera* contain biochemically active constituents, as shown by GC-MS analysis. The antioxidant activity of these extracts may be due to presence of these constituents. These findings emphasize the potential of both extracts as sources of natural antioxidants and antimicrobial agents, further validating their health benefits and traditional medicinal uses.

5. Conclusion

This study underscores the antioxidant potential of ethanolic extracts from *Hibiscus sabdariffa Linn* and *Moringa oleifera* flowers, emphasizing their rich phytochemical composition. The antioxidant activity, assessed through DPPH radical scavenging, hydrogen peroxide scavenging, reducing power, and phosphomolybdate assays, demonstrated that both extracts possess significant free radical scavenging and reducing abilities which may be contributed to the presence of phenolics and flavonoids in the extract as both the extracts have significant amount of total phenolic and flavonoid content.

The GC-MS analysis revealed a diverse range of bioactive compounds in both extracts, including hexadecanoic acid, its esters, and other fatty acid derivatives, which are known for their antioxidant and antimicrobial properties. The compounds such as terpenoids, including ursolic aldehyde, lupeol, α-amyrin, and trimyristin, further highlight the therapeutic potential of these plants. The variability in biological activities observed between the two extracts can be attributed to differences in their phytochemical profiles and environmental factors affecting the plants' growth and development.

The findings validate the traditional medicinal uses of *Hibiscus sabdariffa Linn* and *Moringa oleifera* flowers and highlight their potential as natural sources of antioxidants. These extracts may be valuable in preventing or managing oxidative stress-related disorders and have applications in functional foods, nutraceuticals, and alternative therapies. Further research should focus on elucidating the bioavailability, synergistic interactions, and specific mechanisms of action of these phytochemicals to maximize their health benefits.

6. Future Scope

The present investigation demonstrates significant antioxidant activity and the presence of diverse phytochemicals in the flower extracts of *Hibiscus sabdariffa* and *Moringa oleifera* from the Bhagalpur region. Future studies should aim at isolating and characterizing the individual bioactive compounds identified through GC-MS analysis to elucidate their specific roles. In-vivo studies are essential to validate the observed in-vitro antioxidant effects and to determine the pharmacological safety and efficacy of these extracts. The development of standardized herbal formulations or nutraceuticals based on these findings could offer potential health benefits. Additionally, comparative studies across different geographical regions and seasons may help in identifying the most bioactive variants. Further exploration of molecular mechanisms and synergistic interactions with other natural antioxidants may enhance their application in pharmaceutical and functional food industries.

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