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LC-MS Based Phytochemical Profiling and Antioxidant Activity of Selected Phyto-Extracts

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Abstract:

Plants continue to serve as vital sources of medicinal leads in traditional and modern therapeutics. This study evaluates the phytochemical profile and antioxidant potential of methanolic extracts from three commercially available plant products, Spirulina (S1), Withania somnifera (S2) and Moringa olifera (S3). Qualitative phytochemical screening revealed that all three extracts contained amino acids and proteins, while S1 was rich in flavonoids, saponins and lipids, S2 was rich in cardiac glycosides, tannins and quinones and S3 was rich in alkaloids, carbohydrates, saponins and lipids. LC/MS analysis of methanol fractions identified diverse bioactive metabolites, including flavonoids, peptides, and phenolic derivatives, supporting their roles as redox-active phytoconstituents. Antioxidant proportions were assessed using FRAP, DPPH, and nitric oxide scavenging assays, with gallic acid as the reference standard. S2 consistently exhibited the highest antioxidant activity, with IC50 values closest to those of gallic acid in DPPH and FRAP assays, and strong nitric oxide scavenging. In other hand, S1 and S3 showed comparatively weaker but still significant activity. These findings indicate that the Ashwagandha-based product (S2) harbours a richer array of redox-active phytochemicals and may serve as a promising candidate for further fractionation, structure-based molecular docking, and the development of plant-derived therapeutics for oxidative stress-related metabolic disorders.

Keywords: Phytochemical, Spirulina, Withania somnifera, Moringa, LC-MS, FRAP, DPPH, Gallic acid, IC50, Antioxidant

1. Introduction

Plants remain corner stone medicinal resources in traditional folk medicine worldwide (Sathyaprabha et al., 2010). *Spirulina platensis*, a flavonoid-rich cyanobacterium, offers antioxidant, anti-inflammatory, and anti-cancer effects (Abadi et al., 2022; Ai et al., 2023; Rashmi, 2020), and also contains 60 -70% protein, essential amino acids, carotenoids, vitamins (C, E), and selenium (Ahda et al., 2024; Chei et al., 2020 and Karkal et al., 2023). *Withania somnifera* (Ashwagandha), an ancient Rasayana, combats stress, diabetes, cancer, infections, and arthritis via withanolides (e.g., withaferin-A, withanolide-D), alkaloids, and sitoindosides, promoting rejuvenation and immunity (Dar et al., 2015; Salve et al., 2021; Singh et al., 2011). *Moringa oleifera*, a nutrient-dense tree, yields proteins, phenolics, flavonoids (e.g., quercetin, kaempferol), vitamins, and minerals with antioxidant, antidiabetic, antimicrobial, and cardioprotective activities across its parts (Gupta et al., 2018; Kou et al., 2018).

This study screens phytochemicals in these extracts, links structures to bioactivities, and prioritizes abundant compounds for molecular docking, advancing plant-based therapeutics for metabolic diseases.

2. Materials and methods

2. 1 Procurement of plant products

The plant materials, such as *Spirulina* (S1) powder, Ashwagandha (S2) root powder sourced from HNCO Organics Pvt. Ltd, 36/A, Sumel-5, Asarwa, Ahmedabad, India - 380016. Lic No. GA/713-A and *Moringa olifera* (S3) leaf powder were procured from Organic India Pvt. Ltd., C-5/10, Agro Park, Phase-II, UPSIDC Industrial Area, Kursi Road, Barabanki- 225302, Uttar Pradesh, India. Lic. No. 10018051002605. All extracts were prepared in methanol for all biochemical assays.

2.2 Screening of phytochemicals present in plant material.

The methanolic extract samples were subjected to chemical analysis. A qualitative screening of phytochemicals for the detection of the presence of alkaloids, glycosides, steroids, tannins, saponins, and carbohydrates, etc., was performed using the following methods, as shown in Table 1.

Phytochemicals	Test
Alkaloids	Wagner's test
Carbohydrates	Molisch's test
Cardiac glycosides	Keller Kelliani's test
Flavonoids	Alkaline reagent test
Phenols	Ferric chloride test
Phlobatannins	Precipitate test
Amino acids and proteins	Ninhydrin Test
Saponins	Foam test
Sterols	Liebermann-Burchard test
Tannins	Braymer's test
Terpenoids	Salkowski's test
Quinones	HCl Test
Oxalates	Acetic Acid Test
Fats and oils	Copper Sulphate Test

Table 1: Phytochemical Screening of Samples by using various methods.

2.3 Liquid chromatography-mass spectroscopy (LC-MS) analysis

A Hewlett-Packard 1100 chromatograph (Agilent Technologies, Santa Clara, CA) was used to determine Flavonoid compositions from the methanol extract fractionation. The analysis was performed using a quaternary pump and a diode array detector (DAD). The column is coupled with an MSD Ion Trap XCT mass spectrometer (Agilent Technologies, Santa Clara, CA) equipped with an electrospray ionization interface (ESI).

2.4 Biochemical Assay

2.4.1 FRAP: a biochemical assay for measuring antioxidant capacity

FRAP (Ferric Reducing Antioxidant Power) antioxidants in the sample donate electrons, turning the solution a blue colour, which is measured spectrophotometrically. The FRAP assay was developed by Benzie and Strain (1996) to measure the ferric reducing power of human plasma. Assessing antioxidant capacity in biological fluids (like blood plasma) and food. FRAP is analysed with different concentrations of the methanolic extracts (100-500µg) of samples, which were added in different test tubes, and the volume in each test tube was made up to 0.1mL with methanol. To all the tubes, 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K_3 Fe $(CN)_6$] solution were added. The reaction mixture was vortexed well and then incubated at 50°C for 20 min using a vortex shaker. 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 mins at the end of the incubation. The supernatant was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The coloured solution was read at 700 nm against the blank using a UV-Spectrophotometer.

2.4.2 DPPH radical-scavenging effect assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described in a previous study (Feki et al. 2005). Free radical inhibition, DPPH, in percent (I%) was calculated as follows:

$$I\% = (A_{control} - A_{sample}/A_{control}) \times 100$$

where I is the free radical inhibition, $A_{control}$ is the absorbance of the control reaction (containing all reagents except the studied sample), and A_{sample} is the absorbance of the studied sample. The extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted of inhibition percentage against extract concentration. Different concentrations (0.1 - 0.5 mg) of the samples were taken in the test tubes, and the volume in each test tube was made up to 0.1mL with methanol. To all the tubes, 3mL solution of DPPH (whose absorbance was pre-set to 1) was added and incubated in dark conditions for 15 minutes.

2.4.3 Nitric Oxide assay standard

The percentage inhibition of the extract and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the methanol extracts was calculated using the following standard formula:

$$I\% \text{ inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ - Absorbance of control, A_{sample} - Absorbance of tested samples, and can be compared with the absorbance of standards used. Nitric oxide assay was done by taking a volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate-buffered saline, which was mixed with 1 mL of the different concentrations (0.1 - 0.5 mg) of the samples and incubated at 25°C for 180 minutes. The extract was mixed with an equal volume of freshly prepared Griess reagent (Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use). Control samples without the extracts but with an equal volume of buffer were prepared similarly to the test samples. The absorbance was measured at 546 nm. Gallic acid was used as the positive control.

3. Results

Presence or absence of each phytochemical was recorded for S1, S2 and S3 as shown in Table 2. By using preliminary phytochemical screening and standard qualitative tests. Amino acids and proteins were present in all the samples, while phenols, phlobatannins, sterols, terpenoids, and oxalates were absent. S1 contains flavonoids, saponins, and lipids and S2 has the cardiac glycosides, tannins, and quinones. S3 has reported the presence of alkaloids, carbohydrates, saponins, and lipids.

Phytochemical	Samples		
	S1	S2	S3
Alkaloids (Wagner's test)	-	-	+
Carbohydrates (Molisch's test)	-	-	+
Cardiac glycosides (Keller Kelliani's test)	-	+	-
Flavonoids (Alkaline reagent test)	+	-	-
Phenols (Ferric chloride test)	-	-	-
Phlobatannins (Precipitate test)	-	-	-
Amino acids and proteins (Ninhydrin Test)	+	+	+
Saponins (Foam test)	+	-	+
Sterols (Liebermann-Burchard test)	-	-	-
Tannins (Braymer's test)	-	+	-
Terpenoids (Salkowski's test)	-	-	-
Quinones (HCl Test)	-	+	-
Oxalates (Acetic Acid Test)	-	-	-
Fats and oils (Copper Sulphate Test)	+	-	+

Table 2: Preliminary Qualitative Phytochemical Screening of Samples (**Note:** presence (+) or absence (-)).

In the FRAP assay, gallic acid used as the standard, exhibited an IC50 value of 99.23 mg. S3 showed the highest value of 247.21mg followed by S1, which showed 246.93 mg, whereas S2 showed a lower IC50 value o164.81mg

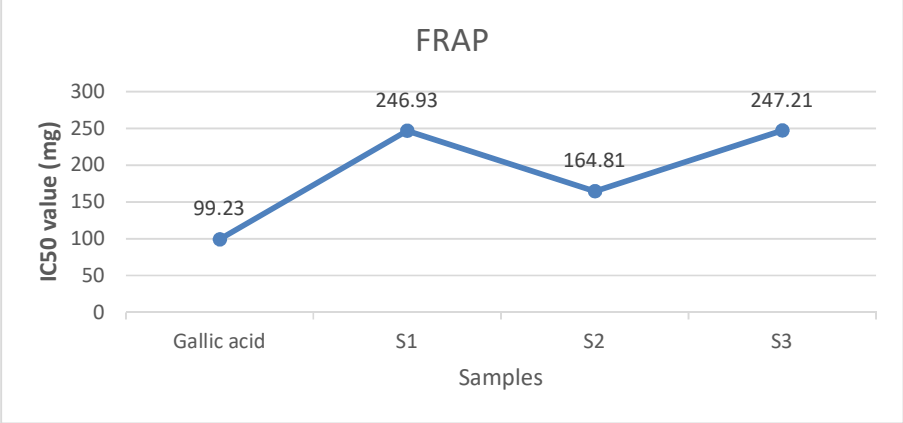

Figure 1. FRAP assay of the phytoextracts with Gallic acid.

In the Nitric oxide (NO) assay, gallic acid, used as the standard, exhibited an IC50 value of 0.097 mg. S1 showed the highest value of 0.162 mg, while S2 decreased to 0.122 mg, followed by S3, which showed an IC50 value of 0.117 mg (Figure 2)

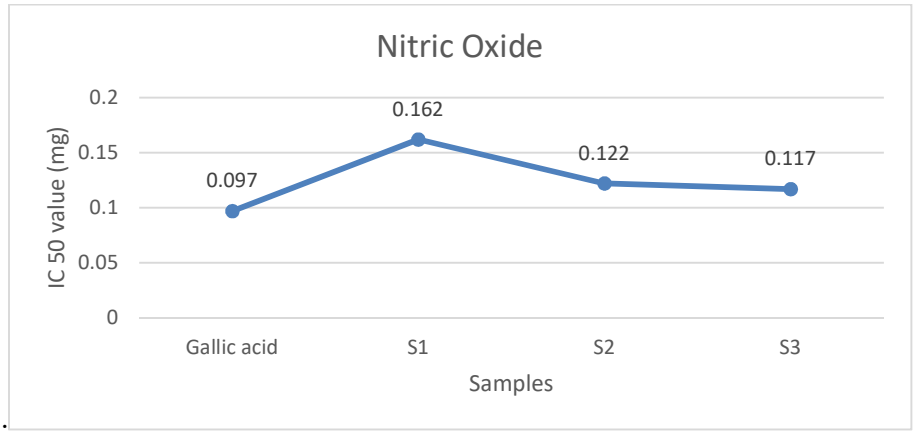

Figure 2. Nitric oxide assay of the phytoextracts with Gallic acid.

In the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, gallic acid, used as the standard, exhibited an IC50 value of 0.8 mg. S3 showed the highest value of 4.487 mg, and a decreased IC50 value of 2.499 mg in S1, further reducing the IC50 value to 0.824 mg in S2 (Figure 3).

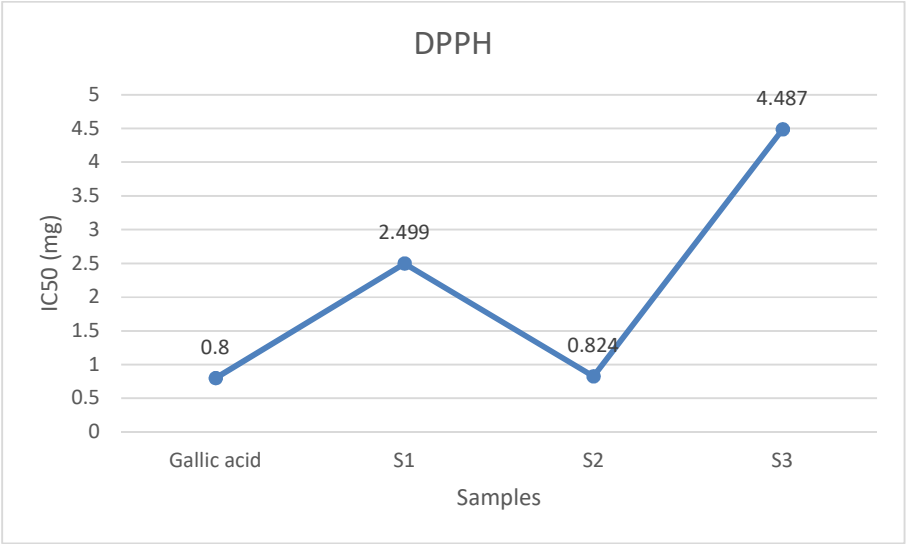

Figure 3. DPPH assay of the phytoextracts with Gallic acid.

4. Discussion

The present study evaluated the phytochemical constituents and antioxidant capacities of methanolic extracts from *Spirulina* (S1), Ashwagandha S2) and *Moringa* (S3) through qualitative screening and free radical scavenging assays (FRAP, DPPH and nitric oxide). The Sample S1 (*Spirulina*) showed the presence of flavonoids, saponins, and lipids, which revealed its metal chelating abilities and the potent antioxidant present in the compound (Abadi et al. 2022 and Shydlovska et al. 2023). The Sample S2 (Ashwagandha) contains amino acids, cardiac glycosides, tannins, and quinones, which are bioactive characteristics and are known for redox-active withanolides like withaferin-A and withanolide-D (Singh et al., 2011). The sample S3 (*Moringa*) contains alkaloids, carbohydrates, saponins, and lipids, which showed antioxidant and antimicrobial properties (Gupta et al., 2018 and Kou et al., 2018).

FRAP assay showed that Ashwagandha (S2) has a stronger ferric-reducing ability than S1 and S3. S2 revealed comparatively higher electron-donating capacity and might be attributed to a higher concentration of polyhydroxylated glycosides and quinones capable of stabilizing the ferric-ferrous complex, correlating with the results of Salve et al. (2021) and Pantami et al. (2020).

In the nitric oxide scavenging assay, S3 exhibited the lowest IC50 after the standard gallic acid, followed by S2 and S1. The variation reflects the involvement of structurally distinct antioxidant mechanisms. While polyphenolics and glycosides in S2 may act through direct competition with oxygen for nitric oxide radicals, alkaloids and saponins in S3 possibly modulate redox homeostasis via electron transfer and metal-chelating pathways (Dar et al., 2015; Shylaja et al., 2022 and Sowmya BH et al., 2020).

The DPPH assay revealed S2 exhibited the lowest IC50 value, compared to S1 and S3, which may be due to its superior hydrogen atom transfer potential. S1 and S3 showed moderate to low activities, underscoring the phenolic and glycosidic composition in S2. This finding aligns with Mishra et al. (2000), who reported that the synergistic presence of withanolides enhances free radical neutralization and oxidative stress mitigation.

5. Conclusion

The comparative analysis of *Spirulina* (S1), Ashwagandha (S2), and *Moringa* (S3) revealed distinct phytochemical profiles and antioxidant capacities. All three phyto-extracts showed antioxidant potential, with Ashwagandha (S2) exhibiting the strongest activity across FRAP, DPPH, and nitric oxide assays, close to that of gallic acid. This superiority is likely due to its rich content of phenolic and glycosidic compounds. The findings suggest that Ashwagandha is a promising source for isolating potent natural antioxidants for further biochemical and therapeutic investigation.

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