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SHORT COMMUNICATION

Phytochemical Characterization and Evaluation of Anti-oxidant Activity of *Ipomoea obscura (L.)*

Durgesh Agase[1*], K. S. Saini[2], M. S. Markam[1], A. S. Kale[3], T. S. Kothe[2], S. Ukey[4], S. Khan[4] and S. Turkar[5]

[1]Govt. J.S.T.P.G. College, Balaghat - 481101, Madhya Pradesh, India; sbt.durgesh@gmail.com
[2]Kamla Nehru Girls College, Balaghat - 481001, Madhya Pradesh, India
[3]Shri Shivaji Science College, Amravati - 444601, Maharashtra, India
[4]Sardar Patel University, Balaghat - 481331, Madhya Pradesh, India
[5]Ravindra Nath Tagore University, Bhopal - 464993, Madhya Pradesh, India

Abstract

Background: Dietary botanicals, including food additives, fruits, and vegetables, have been used for centuries as natural health remedies, but scientific evidence on their preventive properties remains limited. These dietary plants are a significant source of many natural antioxidants. Natural herbal medicines have no side effects on human health so these herbal medicines can be a safe alternative to harmful allopathic medicines. **Aim:** In this study, we evaluated the antioxidant potential of *Ipomoea obscura* leaf extract and profiled the bioactive compounds of the leaf extract. **Methods**: UV-visible spectroscopy, DPPH analysis, and LC-MS analysis. **Results:** UV-visible analysis of water, ethanol, and methanol extracts of *I. obscura* showed maximum absorption at 310.0 nm, 413 nm, and 337 nm. Results of DPPH analysis showed that the aqueous extract (53.80 ± 1.45, R^2 = 0.92) has the maximum antioxidant potential compared to the ethanol and methanol extracts. Phytochemical analysis of the aqueous extract of *I. obscura* revealed several phytochemicals including alkaloids, carbohydrates, phenols, proteins, and saponins. LC-MS data confirmed the presence of 12 bioactive compounds in the aqueous extract of *I. obscura* leaves. **Conclusion:** The study reveals that the aqueous extract of *I. obscura* exhibits high antioxidant potential compared to ethanolic and methanolic extracts and has active secondary metabolites.

Keywords: Antioxidant Potential, Phytochemical Analysis, LC-MS

1. Introduction

Dietary botanicals have been utilized for centuries as natural remedies for various health conditions. These botanicals, consisting of food additives, fruits, and vegetables, offer a holistic approach to promoting human well-being and potentially preventing ailments. However, despite their historical usage, the scientific community still lacks conclusive evidence regarding the preventive properties of dietary plants and their bioactive compounds against various diseases. These dietary plants are a significant source of many natural antioxidants. About 5% of inhaled oxygen is converted to reactive oxygen species ROS during aerobic respiration. ROS are molecules that contain one oxygen with one or more unpaired electrons[1]. Pathogenesis of several diseases such as diabetes, liver disease, nephrotoxicity, cancer, cardiovascular disease, and neurological disorders is attributed to the overproduction of ROS[2]. Antioxidant substances donate an electron to ROS and convert it to a harmless molecule. They could lessen the free radical's energy, prevent radical generation, stop chain reactions, repair damage, and recreate membranes. Antioxidants are either naturally generated (endogenous antioxidants), or externally provided through foods (exogenous antioxidants). Dietary plant substances are a rich

*Author for correspondence

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source of exogenous antioxidants, which play a vital role in assisting endogenous antioxidants in neutralizing oxidative stress.

The morning glory plant *I. obscura*, also called "Laksmana" in *Ayurveda*, is a member of the family Convolvulaceae[3]. This plant is a small climbing vine with specific characteristics such as small cordate leaves and a corolla comprising five fully fused petals. It is indigenous to the Mascarene Islands, Malaysia, northern Australia, Fiji, tropical Asia, and tropical East Africa[4]. It works well for treating acne, open sores, and dysentery. Several species of *Ipomoea* are used to treat gynaecological issues, blood diseases, urinary tract infections, constipation, and female sterility[5]. The plant is also having laxative, psychedelic[6], anticarcinogenic, hepatoprotective, oxytocic, and antioxidant properties[7]. They are also used in rheumatism and fungal infections[8]. In Lambani tribal areas of the Kalaburagi district, *I. obscura* was successfully used to fix animal bones[9]. The antioxidant activity of any dietary botanicals is crucial for the treatment of many diseases or disorders. In the present study, we have explored the antioxidant activity of ethanolic, aqueous, and methanolic extracts of *I. obscura*.

2. Materials and Methods

2.1 Selection of Plant

In the present study, *I. obscura* was selected for phytochemical characterization and evaluation of antioxidant activity. The leaves of plants were collected from Moti Garden, Balaghat, Madhya Pradesh, India. The collected plant was identified in the Department of Botany, Govt. J.S.T.P.G. College, Balaghat. The leaves were selected for the experiment. Leaves are ground in the grinder and air-dried to prepare coarse powder.

2.2 Preparation of Extract

The three different (aqueous, ethanolic, and methanolic) extracts of *Ipomoea obscura* were prepared for the present study. Leaves are ground separately in three solvents using a grinder and air-dried to prepare coarse powder. The coarse powder was subjected to Soxhlet extraction with distilled water, ethanol, and methanol respectively. The dried extract powder was stored in deep freeze until use.

2.3 Determination of Antioxidant Activity

The anti-oxidant activity of each of the three different plant extracts was determined by using the colourimetric DPPH assay[10]. The 10 µL reaction mixture of the test sample, was mixed with 190 µL of methanolic solution of 0.1 mM DPPH radical along with the ascorbic acid as positive control. The mixture was mixed vigorously and incubated at 37°C for 5 min. The absorbance was measured at 517 nm using an ELISA plate reader and free radical scavenging activity, was calculated using the following equation:

$$\%\ \text{free radical scavenging effect} : \frac{[Absorbance\ of\ control(Ac) - Absorbance\ of\ sample(As)]}{Absorbance\ of\ control(Ac)} \times 100$$

2.4 Qualitative Phytochemical Test

Among the three extracts, the most potent antioxidant extract was used for further analysis. The most potent antioxidant extract of *I. obscura* was tested for various components by their specific tests *viz.* Mayer's test, Dragendroff's test for alkaloids, Ferric chloride test for tannins and phenolic compounds; Biuret test, Ninhydrin test for proteins and amino acids; Molisch's test, Benedict's test for carbohydrates and Foam test for saponins[11].

2.5 UV-Vis Spectra Characterization

Preliminary characterization of the different plant extracts was carried out using UV–visible spectroscopy. The spectra scan was monitored by a UV-Vis spectrophotometer from 200 to 700 nm.

2.6 Liquid Chromatography-Mass Spectrometry (LC-MS) Characterization

LC-MS study of the most potent antioxidant extract was used for the determination of bioactive compounds. LC-MS of the plant extracts was carried out at Sophisticated Analytical Instrument Facility, CSIR-Central Drug Research Institute, Lucknow, using a standard optimized method.

2.7 Results

The aqueous extract of leaves of *I. obscura* was successfully prepared and was brownish and gummy in appearance. The same extract was used for further studies.

3. Determination of Antioxidant Activity

The antioxidant capacity of *I. obscura* leaf extracts in aqueous, methanolic, and ethanolic extracts was assessed using the DPPH assay. The ascorbic acid was used as a positive control. The findings of the comparative statistical study indicated that the *I. obscura* aqueous extract had the highest antioxidant potential of 53.80±1.45, with $R^2 = 0.92$ as compared to the methanolic and ethanolic plant extracts. Table 1 and Figures 1, 2 and 3 provide an overview of the findings.

4. Phytochemical Analysis

Aqueous extract of *I. obscura* possessing maximum antioxidant potential was subjected to further studies including preliminary phytochemical evaluation using tube test. After the phytochemical screening, it was found that the aqueous extract of *I. obscura* contains several phytochemicals including alkaloids, carbohydrates, phenols, proteins, and saponins. The results are shown in Table 2.

5. UV-Visible Analysis

Aqueous, ethanolic, and methanolic extracts of *I. obscura* were scanned under UV-visible regions in the range of 200 nm to 700 nm. Variation was found in the peak of different extracts of *I. obscura*. The aqueous, ethanolic, and methanolic extracts of the plant showed maximum absorption at 310.0 nm, 413 nm, and 337 nm respectively.

6. LCMS Analysis

The findings of LC-MS analyses of *I. obscura* aqueous extracts are displayed in Table 3. The peak and retention

Table 1. Antioxidant potential of plant extract using the DPPH assay method

No.	Conc.	EtOH (Mean ±SD)		Aq (Mean ±SD)		MeOH (Mean ±SD)		Ascorbic Acid (Mean ±SD)	
1	0.2 mg	S1	14.40± 1.25	S6	18.14± 2.05	S11	8.36±1.44	S16	20.49±1.56
2	0.4 mg	S2	20.93±1.03	S7	20.87±1.25	S12	17.74±1.52	S17	54.33±1.87
3	0.6 mg	S3	22.20±2.05	S8	22.70±1.96	S13	25.22±3.66	S18	68.06±1.52
4	0.8 mg	S4	31.05±1.87	S9	31.44±0.25	S14	40.64±0.66	S19	79.49±0.98
5	1.0 mg	S5	43.88±1.54	S10	53.80±1.45	S15	46.73±1.87	S20	86.33±1.44
R2		R2 = 0.92		R2 = 0.7946		R2 = 0.984		0.9057	
Y equation		y = 6.908x + 5.768 R2 = 0.92		y = 8.189x + 4.823		y = 9.964x - 2.154		y = 15.684x + 14.688	

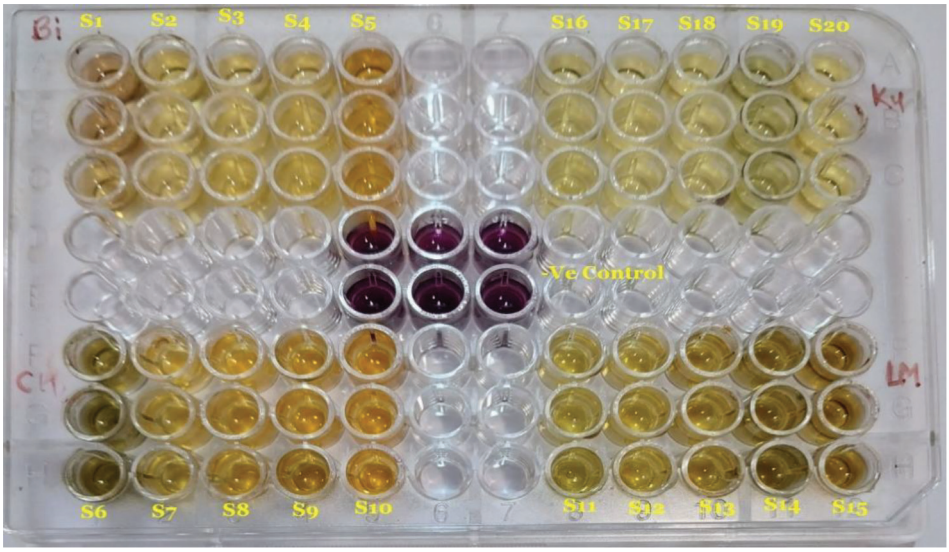

Figure 1. Evaluation of antioxidant potential using DPPH assay.

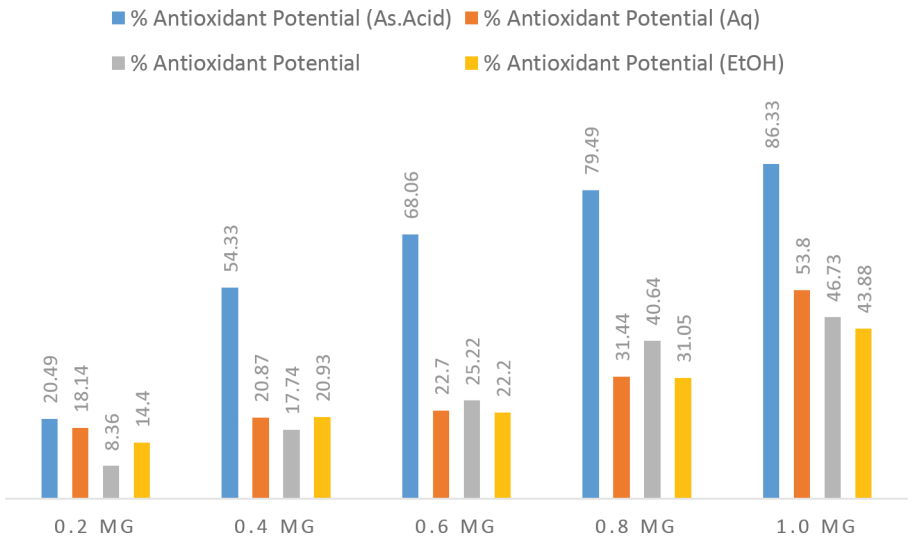

Figure 2. Percent antioxidant potential.

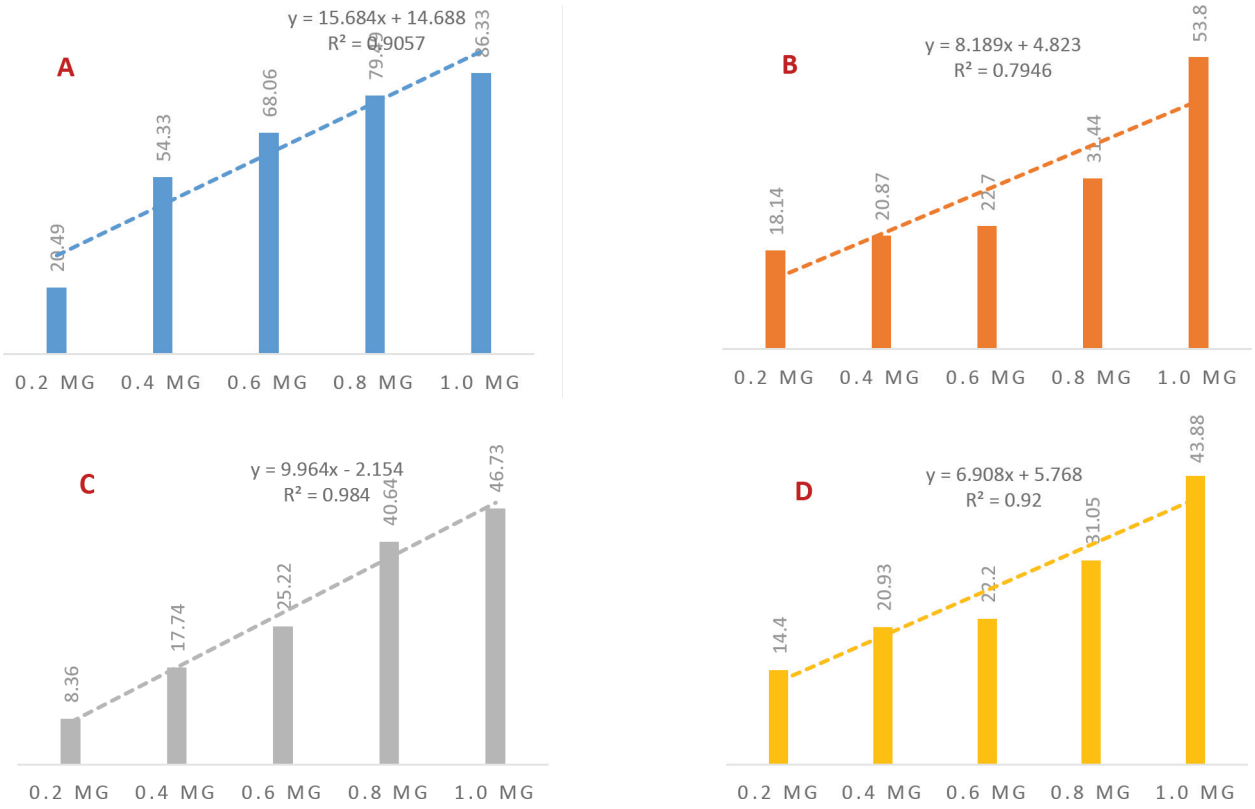

Figure 3. Percent antioxidant potential (A: Ascorbic acid, Aqueous extract, Methanolic extract, Ethanolic extract).

periods revealed the wide spectrum of chemicals present in the extract. The complexity of the chemicals in the plant extract was demonstrated by the large number of peaks. At a particular retention time, several chemicals were found in the extract at high amounts. The aqueous extract chromatogram indicates 12 peaks. Table 3 provides a comprehensive list of the compounds that were identified from the extract that matched the library.

7. Discussion

To make the most efficient and responsible use of the natural resources that are now available, phytochemical

Table 2. Qualitative phytochemical analysis of aqueous extract of *Ipomoea obscura*

S. No.	Phytochemicals	Name of test	Inference
1	Alkaloids	Mayer's test	+
		Dragendorff's Test	+
2	Carbohydrates	Molisch's test	+
		Benedict's test	+
		Fehling's test	+
3	Phenols	Ferric chloride test	+
		Lead acetate test	+
4	Proteins and Amino Acids	Biuret test	+
		Ninhydrin test	+
5	Saponins	Foam test	+
6	Fats and oils	Spot test	-

Table 3. LC-MS analysis of aqueous leaf extract of *I. obscura*

S. N.	RT	Compound	Molecular Formula
1	1.13	3-Methoxy-2,2-dimethyl oxirane	$C_5H_{10}O_2$
2	6.30	Butane-1,2,3,4-tetraol	$C_4H_{10}O_4$
3	15.14	Heptadeane	$C_{23}H_{48}$
4	16.51	Octadecane	$C_{26}H_{54}$
5	22.17	Oleic acid	$C_{39}H_{76}O_3$
6	22.98	Tetracycline	$C_{22}H_{24}N_2O_8$
7	25.73	Ursodeoxycholic acid	$C_{24}H_{40}O_4$
8	26.03	Ethyl iso-allocholate	$C_{26}H_{44}O_5$
9	26.34	Chlortetracycline	$C_{22}H_{23}ClN_2O_8$
10	26.65	Demeclocycline	$C_{21}H_{21}ClN_2O_8$
11	26. 97	2-Cholestanone	$C_{33}H_{50}O$
12	32.39	Lycopene	$C_{40}H_{56}$

screening is crucial for discovering novel sources of chemicals with medical value that are beneficial both therapeutically and industrially[11]. Plants contain a wide range of bioactive compounds that are used in traditional medicine to treat different diseases. In this study, the antioxidant activity of aqueous and ethanolic extracts was evaluated along with phytochemical studies. The aqueous extracts showed potent antioxidant activity at various concentrations. The phytochemical analysis of ethanolic and aqueous extracts of *I. obscura* showed the presence of a variety of phytochemical compounds such as alkaloids, carbohydrates, phenols, proteins, and saponin, while negative results were observed for fat and steroids. In recent years, several experimental studies have elucidated the biological, pharmacological, and antimicrobial properties of the phenolic compounds[12], antiviral, anti-inflammatory, and cytotoxic activity. The phenols and the alkaloids that are present in *Ipomoea* may be linked to traditional medicines used to treat various ailments. Phenols have been extensively studied for their potential health benefits. It has been well-documented that the majority of medicinal plants contain phenolic compounds, which are known to have powerful antioxidant properties, as well as bioflavoronoids[12]. The presence of primary phytoconstituent investigation is supported by the earlier report[13,14]. The presence of various secondary metabolites such as phenolic alkaloids, flavonoids, tannins and glycosides is directly responsible for the potential antioxidant activity of the plant[15,16]. Analysis of primary spectroscopy data helps to understand the chemical functionality of the compound in plant extracts by confirming the presence of several previously reported functional groups. A DPPH test was performed to determine the antioxidant potential of water and ethanol extracts of *I. obscura*. Workers conducted various antioxidant tests to investigate the antioxidant potential of *I. obscura* (L)[17,18]. The antioxidant activity of *I. obscura* was the highest in the aqueous extract compared to other extracts. This suggests that more contribution of saturated compounds in the antioxidant activity of *I. obscura*[4].

8. Conclusion

The results of this study showed that aqueous extracts of *I. obscura* (L.) showed the presence of most of the secondary metabolites in the leaves of the plant. Primary spectroscopic data and LC-MS data confirm the presence of active compounds, functional groups and trace elements in the leaf extract. This study shows that the aqueous extract of *I. obscura* has a high antioxidant potential compared to ethanolic and methanolic extracts.

9. References

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