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LC-MS-Based Metabolite Profiling and Antioxidant Capacity Assessment of *Ipomoea eriocarpa* Extract

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ABSTRACT

The objective of the research was to investigate the phytochemical analysis, total phenolic, total flavonoid, total alkaloid, *in-vitro* antioxidant profile and LC-MS analysis of *Ipomoea eriocarpa* whole plant extract. The process of extraction took place utilizing a Soxhlet apparatus with a hydroalcoholic solvent. An examination of qualitative phytochemicals showed the existence of phenolic, alkaloids, flavonoids, triterpenes, tannins, and unsaturated steroids The study determined the quantities of total phenolics, total flavonoids, and total alkaloids present were 94.6 ± 1.7 mg gallic acid equivalent (GAE)/g sample, 81.25 ± 2.2 mg quercetin equivalent/g, 44.32 ± 2.8 mg atropine equivalent/g. The evaluation of antioxidant properties was conducted using multiple methods, including DPPH assay, ferric reducing-antioxidant power (FRAP), reactive nitrogen oxide, and hydroxyl free radical scavenging method. The IC_{50} values in DPPH assay of standard (ascorbic acid) and sample were found to be 14.04 ± 0.02 and 36.12 ± 0.11 and FRAP scavenging assay of standard (ascorbic acid) and sample were found to be 3.167 ± 0.02 and 1.548 ± 2.75 respectively. The LC-MS (ES+) analysis of extract reported the presence of twelve phytoconstituents along with seven phytoconstituents in LC-MS (ES-). The study concluded that the *I. eriocarpa* hydroalcoholic extract possess antioxidant properties due to the existence of phenols and flavonoids and thus can be a valuable agent to prevent the development of various diseases.

Keywords: Antioxidant activity, *Ipomoea eriocarpa*, Liquid chromatography-Mass spectroscopy, Total alkaloid content, Total flavonoid content, Total phenolic content.

INTRODUCTION

Medicinal plants have significant importance for the health care of local communities as a source of medicine. Since immemorial, plants have played an important part in the human health care system. 422,000 have been documented globally, of which over 50,000 are used medicinally by a large number of people living in rural areas. Approximately 80% of people worldwide utilize herbal remedies for basic healthcare, with emerging nations using them the most[1,2]. Over 4.5 million the use of medicinal plants as part of healthcare has become more common in rural regions owing to the expensive price and negative effects of allopathic pharmaceuticals.

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Oxidative stress is the main consider in growth and progression of diabetes mellitus, cancer, heart disease, neurological disorders, and inflammatory diseases, amid other conditions. The body has a sophisticated system of defense against free radicals that includes both enzymatic and nonenzymatic pathways. In a healthy condition, these pathways sustain a constant balance between prooxidants and antioxidants, which promotes overall health[3]. Typically, several synthetic antioxidant chemicals including propyl gallate (PG), butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) are used to treat oxidative stress. These synthesised antioxidant chemicals have been linked to negative consequences despite their use[4]. In light of alternative and complementary approaches, medicinal plants have a greater potential of offering effective, secure, reasonably priced, and conveniently accessible treatments for diseases associated with oxidative stress[5].

Ipomoea eriocarpa commonly known as tiny morning glory is found in tropical Asia, northern Australia, Madagascar, South Africa, Egypt, among other places in tropical Africa and southern parts of India[6]. According to a phytochemical study, the plant's (methanolic extract) contains phenols, flavonoids, phytosterols, and alkaloids[7,8]. The traditional uses of *I.eriocarpa* (methanolic and petroleum ether extract) includes migraines, joint inflammation, seizures, open sores, and high fever. The preclinical study of *I. eriocarpa* has recently confirmed protective effects on brain[9], antioxidant capabilities[10], inhibition of secretions[11], pain relief[12], antipyretic[13], action against worms[14], antimicrobial effects[14], as well as benefits from arthritis, diabetes and kidney stones prevention[8,15].

Since no antioxidant activity was performed on hydroalcoholic extract therefore, *I.eriocarpa* whole plant hydroalcoholic extract was used to assess *in-vitro* antioxidant activity, its quantitative estimation of phytochemicals followed by LC-MS.

MATERIAL AND METHODS

Chemical and reagents

The following chemicals and reagents were utilized: gallic acid, quercetin, atropine, DPPH, BCG and Folin ciocalteu reagent were procured from loba chemie Pvt. Ltd., Sigma-Aldrich, Sisco research laboratories Pvt. Ltd., Central drug house Pvt. Ltd. And all the chemical and reagents were of standard analytical grade.

Collection and authentication of plant

The mature complete plant material of *I.eriocarpa* were collected from the Barkagao, Hazaribag District, Jharkhand (23°85'31.10" N latitude, (85°20'58.51 E longitude and 610m Altitude), India. The voucher specimen (KM81123) was prepared, submitted and authenticated by Dr. P. Santhan Botanist, Taxonomist, Jharkhand.

Preparation of Plant extract

All adhering foreign matter was removed from the *I. eriocarpa* plant material after it was thoroughly cleansed. Following a clean water wash and three to four weeks of under-shade drying, the plant material was coarsely powdered using a mechanical blender. All of the powdered material was stored in dry, sterile bags. About 25 g of powdered material were defated using petroleum ether (40°C-60°C). After defatting the powdered material was used for the extraction with hydroalcohol (3:7) for 26 hours[16]. The extract was maintained at room temperature for drying and concentrated extract was utilized for the preliminary phytochemical tests to analyze the total quantities phenols, flavonoids, alkaloids, in-vitro antioxidant profile, and LC-MS. The formula utilized to determine the extract yield % was as follows:

$$Yield\ \% = \frac{Weight\ of\ solvent\ free\ extract\ (g)}{Dried\ extract\ weight} \times 100$$

Preliminary phytochemical screening

The initial screening for alkaloids, flavonoids, proteins, amino acids, glycosides, tannins, carbohydrates, phenolic, and, steroidal compounds were carried out for hydroalcoholic extract of *I.eriocarpa* by using accepted techniques for its authentication and verification[17].

Assessment of total phenolic content

The assessment of complete phenols in *I. eriocarpa's* extract was estimated using the Folin-Ciocalteu technique[18,19]. Gallic acid (25 mg) was mixed in distilled water in order to produce a standard gallic acid mixture. The extract was dissolved in different concentrations, and flasks were filled with each concentration. The flasks were then adjusted to 25 milliliters and a UV spectrophotometer

(UV-Shimadzu 1900i) was utilized to record the absorbance at 750 nm after 90 min of incubation.

Assessment of total flavonoid content

The complete flavonoid in the extract was evaluated utilizing the Aluminium chloride colorimetric technique. A standard solution of 1 mg/mL was produced by dissolving 25 mg of quercetin in methanol. A 100 mg/mL solution was obtained by further dilution. A mixture of potassium acetate, methanol, aluminium chloride, and water was added to the extracts. A standard curve for the flavonoid quercetin was plotted using different concentrations.[20]

Determination of total alkaloid content
Standard Preparation

Aliquots of the atropine standard solution were quantified and transferred to several funnels. Bromocresol green (BCG) and pH phosphate buffer solutions were introduced, agitated with chloroform, and subsequently collected in a 10 mL flask. The volume was adjusted, and the compound's absorbance in chloroform was measured at 470 nm using a blank without atropine.

Sample Preparation

The sample extract was solubilized in 2 N hydrochloride, filtered, as well as rinsed with chloroform. The solution was neutralized with NaOH, BCG solution, and phosphate buffer. The complex was extracted using chloroform, collected, and diluted. The absorbance in chloroform was determined at 470 nm, after collecting and dilution. The process involved a thorough shaking and careful handling of the mixture.[21]

Determination of *In-vitro* antioxidant assay
DPPH scavenging method

The study involved introducing different stock solutions of a test chemical to a 0.1 mM solution of DPPH within a 96-well plate. The reaction was conducted in triplicate, with untreated wells as the control and blank wells as the blank made with 0.2 millilitres of DMSO/Methanol along with 5 µL of the sample at varying doses. The plate was incubated for half an hour, and decolorization was assessed at 517 nm. The reaction mixture containing 20 µL of deionized water was designated as the control. The value of IC_{50} was calculated using Graph Pad Prism 6 software[22]. The scavenging action was calculated by formula:

$$\frac{A(Control) - A(Sample)}{A(Control)} \times 100$$

Ferric reducing-antioxidant power (FRAP) assay

The study involved adding different concentrations of a test compound and standard (Ascorbic Acid) to a solution of sodium phosphate buffer and potassium ferricyanide. Twenty minutes of incubation at 50°C followed the vortexing of the mixture. To the mixture was added deionized water, 0.1% ferric chloride, and 10% trichloroacetic acid after incubation. For this experiment, a microplate reader was used to read the colored solution at 700 nm. The IC_{50} was then computed using the Graph Pad prism 6.0 program. The following equation was taken to determine the scavenging activity.

$$\frac{A(Control) - A(Sample)}{A(Control)} \times 100$$

Liquid Chromatography-Mass Spectroscopy (LC-MS)

The LC-MS of the sample extract was performed from CSIR-CDRI, Lucknow using a Waters Xevo TQD triple quadrupole mass spectrometer hyphenated with a waters acquity H-class UPLC/PDA system. The two function test were performed i.e., ES+ and ES- with a mass range of 150 to 2000nm for about 40 minutes. The temperature of the column was established at 35°C. The pre-injection wash duration was 0 seconds, whereas the post-injection wash duration was 6 seconds with an injection volume of 2 µL. UPLC eLambda 800 nm was used as detector. Following a comparison with those found in the NIST computer library, which is connected to the device, the phytoconstituents were identified and reported.

Statistical Analysis

GraphPad Prism 6 (Graph Pad Software, Inc., USA) was conducted for statistical investigation with values presented as mean ± standard deviation (SD).

RESULTS

Percentage yield of extract

The hydroalcoholic extraction of *I. eriocarpa* showed percentage yield of 21.96%.

Preliminary phytochemical test

A phytochemical analysis of a freshly made hydroalcoholic extract of *I. eriocarpa* was conducted to determine the amount of various kinds of metabolites responsible for the plant's antioxidant properties. Phenolic compounds, alkaloids, flavonoids, triterpenes, tannins, and unsaturated steroids were present in *I. eriocarpa* as shown in Table 1.

Table 1: Phytochemical analysis of hydroalcoholic extract of *I.eriocarpa*

Sr. No	Test	Result
1	Test for Carbohydrates	
	Molish's Test	Absent
2	Test for Reducing sugars	
	Fehling's Test	Absent
	Benedict's Test	Present
3	Test for Hexose Sugars	
	Selwinoff's Test (for ketohexose like fructose)	Absent
	Cobalt-chloride Test	Absent
4	Test for non-reducing polysaccharides (Starch)	
	Iodine test	Absent
5	Test for Proteins	
	Biuret test (General test)	Absent
	Million's test (for proteins)	Absent
	Xanthoprotein test (for protein containing tyrosine or tryptophan)	Absent
	Test for protein containing Sulphur	Absent
6	Test for Amino acids	
	Test for tyrosine	Absent
7	Test for Steroid	Present
	Salkowski Reaction	
8	Test for glycosides	Present
	Test for deoxysugars(Keller- killiani test)	
9	Test for Saponin Glycosides	
	Foam test	Absent
10	Test for Alkaloids	
	Dragendroff's test	Present
	Mayer's test	Present
	Wagner's test	Absent
11	Test for Flavonoids	Present
	Alkaline reagent test	
	Shinoda Test	Absent
12	Test for Tannins and Phenolic compounds	
	5% $FeCl_3$ solution	Present
	Lead acetate solution	Absent
	Gelatin solution	Absent
	Potassium dichromate	Absent

Total phenolic, flavonoid, alkaloid content

The quantities of phenols, flavonoids, and alkaloids present in hydroalcoholic extract was estimated as shown in Fig. 1, 2 and 3. The values represent the average of three biological duplication and expressed as mean ± standard deviation as depicted in Table 2.

Table 2: Total phenolics, total flavonoids and total alkaloid content of hydroalcoholic extract of *I.eriocarpa*

Test	Content
Total phenolic content	94.6 ± 1.7[a]
Total flavonoid content	81.25 ± 2.2[b]
Total alkaloid content	44.32 ± 2.8[c]

a mg gallic acid equivalent (GAE)/g, b mg quercetin equivalent/g, c mg atropine equivalent/g. Values are means of 3 replicates.
All values are stated as Mean ± Standard deviation (SD)

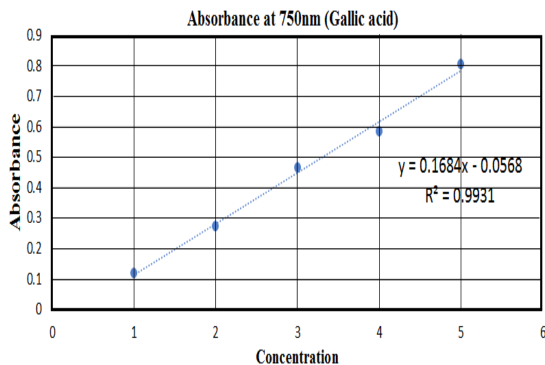

Fig. 1. Calibration curve of gallic acid at different concentrations

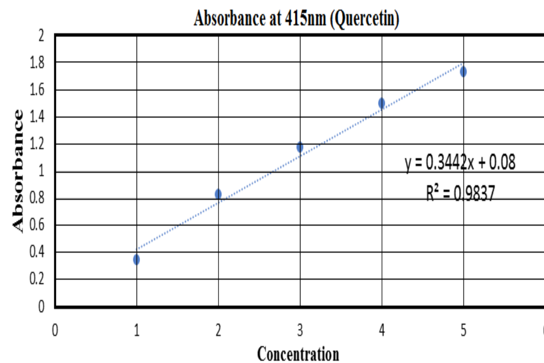

Fig. 2. Calibration curve of quercetin at different concentrations

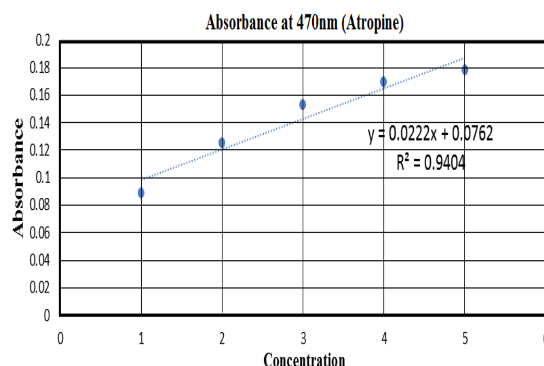

Fig. 3. Calibration curve of atropine at different concentrations

In-vitro antioxidant activity

DPPH scavenging activity

DPPH scavenging assay was performed in samples and 50% inhibitory concentration (IC_{50}) was calculated. The standard compound (ascorbic acid) concentration range was selected from i.e., 0 to 50 µg/mL as shown in Fig. 4 and for sample the concentration was taken from i.e., 0 to 1000 µg/mL as shown in Fig. 5. As calculated in Table 4 the IC_{50} values in DPPH scavenging assay of standard (ascorbic acid) and sample were found to be 14.04 ± 0.02 and 36.12 ± 0.11. The *I. eriocarpa* extract sample (KM17) exhibited a significant DPPH scavenging anti-oxidant activity as represented in Table 3.

Table 3: Percentage scavenging of *I. eriocarpa* extract in DPPH assay

Extract concentration (µg/mL)	Final Replicate values				%Scavenging		
	1	2	3	4	Mean	SD	N
0	0.4939	2.1104	-0.4041	-2.2002	0.00	1.7983	4
1	1.6614	0.0449	-0.4939	0.0449	0.31	0.9333	4
10	13.6955	11.5401	11.5401	12.6178	12.35	1.0318	4
50	69.0166	62.5505	67.2204	69.3758	67.04	3.1385	4
100	77.5482	78.2667	79.1648	79.7036	78.67	0.9546	4
250	81.9488	82.3080	82.8468	83.0264	82.53	0.4946	4
500	83.9245	82.8468	84.1041	83.2061	83.52	0.5934	4
1000	84.4634	88.2265	84.2837	84.6430	84.51	0.1719	4

SD= Standard deviation
N= No. of replicate values

Table 4: IC_{50} value, Hillsope, Degree of freedom, R square and absolute sum of squares value of ascorbic acid and *I.eriocarpa* (KM17) for DPPH scavenging activity

Samples	IC_{50}	Hill Slope	Degrees of Freedom	R square	Absolute Sum of Squares
Ascorbic Acid	14.04 ± 0.02	1.281	5	0.9913	52.54
I.eriocarpa (KM17)	36.12 ± 0.11	1.002	5	0.9412	463

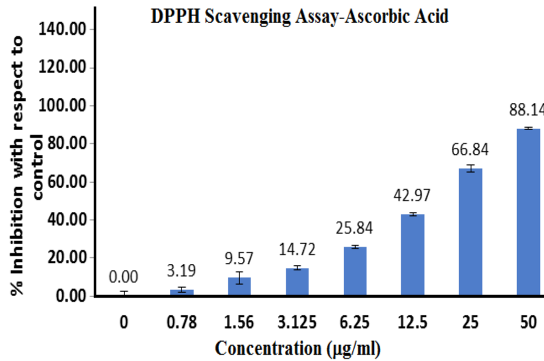

Fig. 4. % of inhibition of DPPH scavenging activity of ascorbic acid at different concentrations

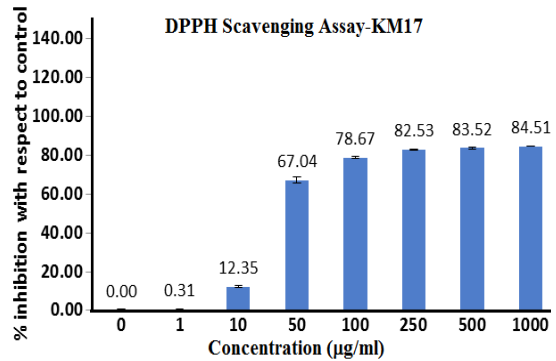

Fig. 5. % of inhibition of DPPH scavenging activity of *I.eriocarpa* at different concentrations

Ferric reducing-antioxidant power (FRAP) assay

FRAP method was estimated in samples and 50% inhibitory concentration (IC_{50}) was calculated for *I.eriocarpa* and ascorbic acid (standard). The amount of ascorbic acid ranged from i.e., 0 to 50µg/mL as shown in Fig. 6 and for sample the concentration was taken from i.e., 0 to 1000 µg/mL as indicated in Fig. 7. As calculated in Table 6 the IC_{50} values in FRAP assay of standard (ascorbic acid) and extract (KM17) were found to be 3.167 ± 0.02 and 1.548 ± 2.75. I.eriocarpa extract possess a significant antioxidant activity through FRAP assay as shown in Table 5.

Table 5: Percentage scavenging of *I. eriocarpa* extract in FRAP assay

Extract concentration (µg/mL)	Final Replicate values				%Scavenging		
	1	2	3	4	Mean	SD	N
0	1.4184	-7.0922	-1.41844	7.0921	0.00	5.9054	4
1	36.87943	31.2056	51.06383	12.76596	32.9787	15.8533	4
10	9.219858	37.5886	94.32624	179.4326	80.1418	75.0567	4
50	158.8652	139.0071	195.7447	175.8865	167.3759	24.1829	4
100	339.0070	323.4043	307.8014	354.6099	331.2057	20.1431	4
250	629.7872	696.4539	656.7376	635.461	654.6099	30.2119	4
500	733.3333	781.5603	719.1489	680.8511	728.7234	41.6189	4
1000	779.4326	707.0922	664.539	595.0355	686.5248	77.2626	4

SD= Standard deviation
N= No. of replicate values

Table 6: IC_{50} value, Hillsope, Degree of freedom, R square and absolute sum of squares value of ascorbic acid and *I.eriocarpa* (KM17) for Ferric reducing-antioxidant power (FRAP) assay

Samples	IC_{50}	HillSlope	Degrees of Freedom	R square	Absolute Sum of Squares
Ascorbic Acid	3.167 ± 0.02	3.769	2	0.992	45.94
I.eriocarpa (KM17)	1.548 ± 2.75	1.751	2	-0.1286	58321

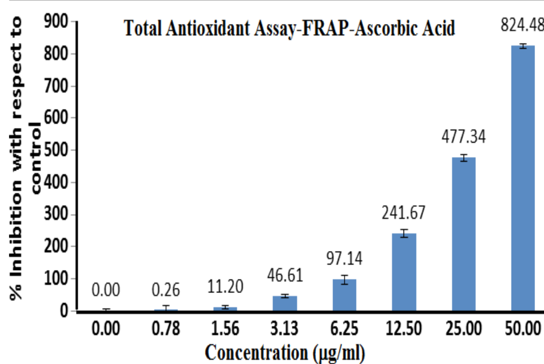

Fig. 6. FRAP radical scavenging assay antioxidant activity of ascorbic acid at different concentrations

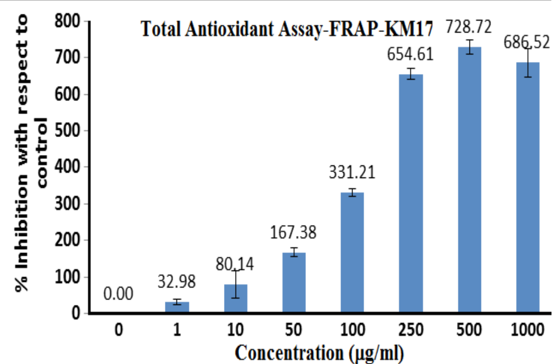

Fig. 7. FRAP radical scavenging assay antioxidant activity of *I. eriocarpa* at different concentrations

LC-MS (ES+) of the extract

The LC-MS (ES+) analysis of *Ipomoea eriocarpa* reported the existence of twelve phytoconstituents as shown in Fig. 8. The major phytoconstituents found in LC-MS (ES+) are 1,2,3,7, 8-Pentachlorodibenzofuran, Methoxyacetamide, N,N-diheptyl-, Hydroxychloroquine, Testosterone, TBDMS derivative, Acebutolol, Spiro[4.5]decane, 7-hexadecyl-, Octanoic acid, morpholide, 7-Tetradecyne, 4-di-n-Butylaminobutanol-1, Benzenemethanol, α-[1-(ethylmethylamino) ethyl]-, [R-(R*,S*)]-, 4-Quinolinol,4-ethenyl-1-ethyldecahydro-2-methyl-(2, 4, 4a, 8a) and Ferruginol as represented in Fig. 9. The interpretation of existing compound according to LC-MS (ES+) spectra was presented in Table 7.

Table 7: The LC-MS (ES+) interpretation of *I.eriocarpa* with its chemical name, molecular formula and chemical structures

Ranking	Retention Time [LC-MS (ES+)]	Molecular Mass	Chemical Name	Molecular Formula	Chemical Structure (Figure)
1	27.41	337.86	1,2,3,7,8-Pentachlorodibenzofuran	$C_{12}H_3Cl_5O_2$	A
2	15.52	285.27	Methoxyacetamide, N,N-diheptyl-	$C_{17}H_{35}NO_2$	B
3	14.81	335.18	Hydroxychloroquine	$C_{18}H_{26}ClN_3O$	C
4	22.94	402.30	Testosterone, TBDMS derivative	$C_{25}H_{42}O_2Si$	D
5	16.20	336.2	Acebutolol	$C_{18}H_{28}N_2O_4$	E
6	30.07	362.39	Spiro[4.5]decane, 7-hexadecyl-	$C_{26}H_{50}$	F
7	2.34, 5.03, 7.88, 38.26	213.17	Octanoic acid, morpholide	$C_{12}H_{23}NO_2$	G
8	36.53	194.20	7-Tetradecyne	$C_{14}H_{26}$	H
9	35.55	201.21	4-di-n-Butylaminobutanol-1	$C_{12}H_{27}NO$	I
10	16.92	193.15	Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-,[R-(R*,S*)]-	$C_{12}H_{19}NO$	J
11	12.48	223.23	4-Quinolinol,4-ethenyl-1-ethyldecahydro-2-methyl-(2 ,4 ,4a ,8aβ)	$C_{14}H_{25}NO$	K
12	9.0	286.23	Ferruginol	$C_{20}H_{30}O$	L

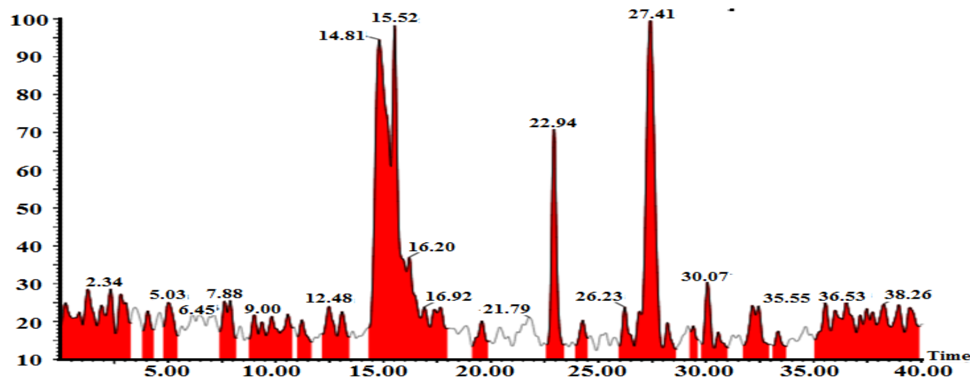

Fig. 8. The liquid chromatography-mass spectra (ES+) of various compounds present in *I. eriocarpa* with its retention time performed from CSIR-CDRI, Lucknow

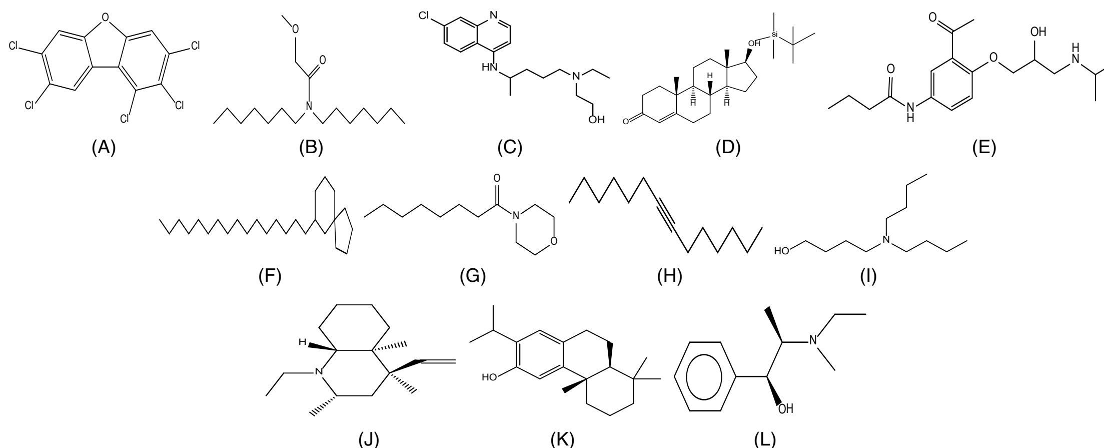

Fig. 9. Chemical structures of phytoconstituents found in *I. eriocarpa* in LC-MS (ES+) LC-MS (ES-) of the extract

The LC-MS (ES-) analysis of *Ipomoea eriocarpa* reported the existence of seven phytoconstituents as shown in Fig. 10. The major phytoconstituents found in LC-MS (ES-) are 8-Heptadecanol, 5-Eicosene, (E)-, Cholecalciferol tert-butyldimethylsilyl ether (TBDMS) derivative, Vinclozolin, 3-Eicosyne, Octacosyl pentafluoropropionate and 9-Octadecenoic acid, (E)-, TMS derivative as represented in Fig. 11. The interpretation of existing compound according to LC-MS (ES-) spectra was presented in Table 8.

Table 8: The LC-MS (ES-) interpretation of I.eriocarpa with its chemical name, molecular formula and chemical structures

Ranking	RetentionTime [LC-MS (ES-)]	Molecular Mass	Chemical Name	Molecular Formula	Chemical Structure (Figure)
1	24.79	256.28	8-Heptadecanol	$C_{17}H_{36}O$	M
2	21.94	280.31	5-Eicosene, (E)-	$C_{20}H_{40}$	N
3	27.86	498.43	Cholecalciferol TBDMS derivative	$C_{33}H_{58}OSi$	O
4	30.66	284.50	Vinclozolin	$C_{12}H_9Cl_2NO_3$	P
5	19.51	278.30	3-Eicosyne	$C_{20}H_{38}$	Q
6	15.53	556.43	Octacosyl pentafluoropropionate	$C_{31}H_{57}F_5O$	R
7	1.29	354.30	9-Octadecenoic acid, (E)-, TMS derivative	$C_{21}H_{42}O_2Si$	S

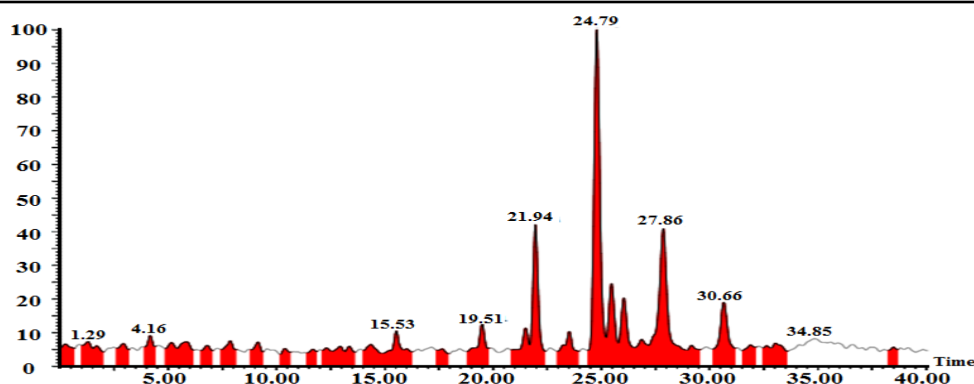

Fig. 10. The liquid chromatography-mass spectra (ES-) of various compounds present in *I. eriocarpa* with its retention time performed from CSIR-CDRI, Lucknow

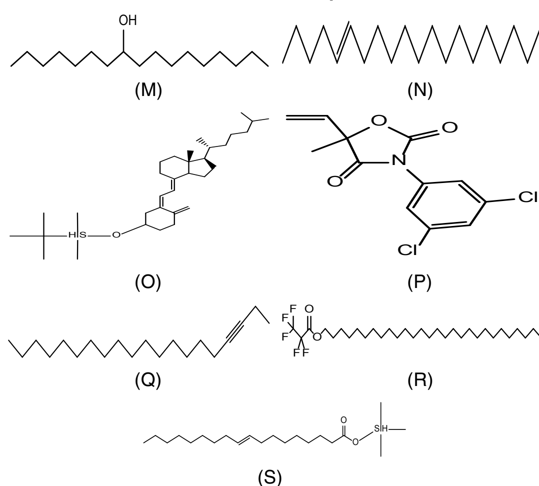

Fig. 11: Chemical structures of phytoconstituents found in *I. eriocarpa* in LC-MS (ES-)

DISCUSSION

The biological actions of medicinal plants, such as their anti-inflammatory, antibacterial, and antioxidant properties, are attributed to their abundance of secondary plant metabolites and important phytochemicals.

The hydroalcoholic extract of *I. eriocarpa* was phytochemically analyzed for several primary and secondary metabolites. Phenols, alkaloids, flavonoids, triterpenes, tannins, and unsaturated steroids are the most significant categories of phytochemicals present in *I. eriocarpa*. The extract lacked saponins and proteins as observed in Table 1. The lack of proteins along with amino acids, and saponins under investigation has also been documented in earlier research 16,23. Alkaloids, flavonoids, flavons, volatile oils, coumarins, steroid glycosides, sterols, and triterpenes were found in several further studies examining the phytoconstituents of various plant sections of *I. eriocarpa*[14,16,23] Numerous additional substances, including tannins and steroids, have strong antibacterial properties[24,25]. Terpenes are antibacterial compounds that work by weakening

the walls of microorganisms' cells and surrounding tissue. They function as antidiabetic and anticancer drugs as well[26]. As a result, each phytochemical has a unique set of characteristics related to biology, including antibacterial, anti-inflammatory, antioxidant, antiplasmodial, and anticancer properties[27]. Phenolic chemicals are another important class of phytoconstituents. These vary from simple phenolic compounds to more complex tannins and are regarded as the most abundant aromatic secondary metabolic products found in plants[28].

TPC of the hydroalcoholic extract of *I. eriocarpa* was recognised by the Folin Ciocalteu reagent with gallic acid serving as a standard, as depicted in Table 2. Phenolic substances have the ability to behave as antioxidants due to their redox properties[29]. The TPC can be used as an efficient method of screening for antioxidants as the hydroxyl groups in them aid them scavenge free radicals. TFC was assessed by aluminium chloride colorimetric technique and quercetin was used as a standard compound. Flavonoids, a class of secondary metabolites found in plants, comprise flavones, flavanols, as well as condensed tannins. However, their antioxidant properties are dependent on free OH groups, especially 3-OH. Total alkaloid content was determined using NaOH, BCG solution, and phosphate buffer and atropine was used as a standard compound Alkaloids are physiologically active substances that plays a most important role as a natural antioxidant. The isoquinoline alkaloids are used in various pharmacological and medicinal approaches.

One of the most used techniques for determining antioxidant potential *in-vitro* is the DPPH method[30,31]. Radical chain reactions function as a prevalent process that causes lipid peroxidation. Radical scavengers enhance the durability and nutritional value of food items by terminating peroxidation reactions in chains[32]. Radical scavenger molecules immediately engage with peroxide radicals and rapidly neutralize them. Free radical scavenging is a technique in which antioxidants directly impede lipid peroxidation. This approach is a common, extensively utilized, and very efficient methodology in research of antioxidant activity. The elimination of radicals is crucial because to the detrimental consequences of reactive oxygen species in dietary and pharmaceutical systems. The IC_{50} values of ascorbic acid and extract depicted that ascorbic acid was potent than extract.

The blue-colored complex is produced when the antioxidant chemicals convert the ferric form into the ferrous form. Reducing power is connected to antioxidant activity and has the potential to significantly lower it. In order to function as both primary and secondary antioxidants, Compounds exhibiting reducing power demonstrate their role as electron donors and have the ability to lower the oxidised precursors of lipid peroxidation processes[33]. Based on the study's findings, significant antioxidant (Ferric reducing-antioxidant power) activity were observed in both extract and ascorbic acid. Comparing the IC_{50} values, both sample and standard exhibited antioxidant activity but *I. eriocarpa* extract showed more potent antioxidant activity than ascorbic acid.

The LC-MS (ES+) analysis of *Ipomoea eriocarpa* reported the existence of twelve phytoconstituents which is represented in Table 7 along with seven phytoconstituents in LC-MS (ES-) which is represented in Table 8. The major phytoconstituents found in LC-MS (ES+) are 1,2,3,7,8-Pentachlorodibenzofuran, Methoxyacetamide, N,N-diheptyl-, Hydroxychloroquine, Testosterone, TBDMS derivative, Acebutolol, Spiro[4.5] decane, 7-hexadecyl-, Octanoic acid, morpholide, 7-Tetradecyne, 4-di-n-Butylaminobutanol-1, Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-, [R-(R*,S*)]-, 4-Quinolinol,4-ethenyl-1-ethyldecahydro-2-methyl-(2, 4, 4a, 8a) and Ferruginol as shown in Fig. 9. The liquid chromatography-mass spectra (ES+) with its retention time of the following compounds present in *I.eriocarpa* is illustrated in Figure 8.

1,2,3,7,8-Pentachlorodibenzofuran, Hydroxychloroquine, Testosterone, TBDMS derivative, Acebutolol, Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-, [R-(R*,S*)]-, 4-Quinolinol,4-ethenyl-1-ethyldecahydro-2-methyl-(2 ,4 ,4a ,8a) and Ferruginol are the seven phenolic compound which are reported in LC-MS ES(+). Methoxyacetamide, N,N-diheptyl- is a amide group, Spiro[4.5]decane, 7-hexadecyl- is a bicyclic compound, Octanoic acid, morpholide is an acyl functional group, 7-Tetradecyne is a fatty alkyne, 4-di-n-Butylaminobutanol-1 is an aliphatic alcohol, The major phytoconstituents found in LC-MS (ES-) are 8-Heptadecanol, 5-Eicosene, (E)-, Cholecalciferol TBDMS derivative, Vinclozolin, 3-Eicosyne, Octacosyl pentafluoropropionate and 9-Octadecenoic acid, (E)-, TMS derivative as shown in Fig. 11. The liquid chromatography-mass spectra (ES-) with its retention time of the following compounds present in I.eriocarpa is illustrated in Figure 10.

8-Heptadecanol, 5-Eicosene, (E), Octacosyl pentafluoropropionate and 9-Octadecenoic acid, (E)-, TMS derivative are fatty acid compounds, Cholecalciferol TBDMS derivative is a vitamin D, Vinclozolin is a fungicide, 3-Eicosyne is a fatty alkyne.

CONCLUSION

The current investigation revealed that *I.eriocarpa* complete plant extract contained Phenols, alkaloids, flavonoids, triterpenes, tannins, and unsaturated steroids. Among them, total polyphenols were in higher concentration and exhibited higher antioxidant activity, making it a possible therapeutic candidate for treating various diseases. Antioxidants potentially guard against illnesses triggered by free radicals by helping to neutralize free radicals, which are the primary reason of inflammatory disorders. Thus, *I.eriocarpa* possesses the significant DPPH scavenging and FRAP activity. The LC-MS (ES+) detected 12 chemical compounds and LC-MS (ES-) illustrated the presence of 7 chemical compounds. Thus, *I.eriocarpa* may be further explored to do more research on the isolation of the plant's bioactive elements as well as *in-vivo* investigations utilizing animal models to examine the plant's possible pharmacological activities. Clinical studies can be further explored to cure various diseases for the future perspective of the expansion of the global herbal market and evaluating their toxicity and adverse drug reactions.

Abbreviations

Ipomoea eriocarpa, PG-propyl gallate, BHA-butylated hydroxyanisole, BHT-butylated hydroxytoluene, BCG-Bromocresol green, TPC-Total phenolic content, TFC- Total flavonoid content, TAC-Total alkaloid content, DPPH-2,2-diphenyl-1-picrylhydrazyl, FRAP-Ferric reducing-antioxidant power, IC_{50}-Inhibitory concentration at 50%, LC-MS-Liquid Chromatography-Mass Spectroscopy, PDA- Photodiode Array, NIST-National Institute of Standards and Technology, MCT-medium chain triglycerides, CSIR-CDRI-Council of Scientific & Industrial Research-Central Drug Research Institute-SD-standard deviation, ES-Electron spectroscopy

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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