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Phytochemical Analysis, in-vitro Antioxidant and Cytotoxicity capacity of *Ipomoea campanulata.*

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ABSTRACT

Ipomoea campanulata, a large shrubby climber plant collected from western ghat region covered under district Pune, Maharashtra to analyze antioxidant and cytotoxicity capacity of its extracts. The total phenolic content measured as gallic acid standard equivalent was found to be 0.440 ± 0.09 mg/ml and 0.380± 0.08mg/ml for methanol and ethyl acetate extract respectively. Flavonoid content was found to be 0.071 ± 0.002 mg/ml and 0.057± 0.002 mg/ml for methanol and ethyl acetate extract as Quercetin standard equivalent. The antioxidant potential of extracts was determined by employing DPPH Scavenging assay and Nitrous oxide radical scavenging assay. The highest % inhibition for DPPH scavenging (68.75 ± 1.08%) and Nitrous oxide radical scavenging (60.93 ± 6.93%) showed by methanolic extract and ethyl acetate extract of I. campanulata respectively. MTT assay showed that the methanolic extract is toxic to HepG2 and MCF 7 cells and their % inhibition are56.32% and 65.99% respectively. The study shows that the extracts of I. campanulata have strong antioxidant and cytotoxic capacity against HepG2 and MCF7 cells.

KEYWARDS

Ipomoea campanulata, Phytochemical, Antioxidant, Cytotoxicity.

1. INTRODUCTION

In the plants, Convolvulaceae family contains nearly 1650 species from which approximately 500-600 species comprises by the genus *Ipomoea*. Hence, large numbers of species are from genus *Ipomoea* within the Convolvulaceae family [1]. The plants with heart-shaped leaves and funnel-shaped flowers under the twining or climbing woody or herbaceous plants dominate this genus. The most species of this genus *Ipomoea* are in the tropical region and some are in the temperate zones [2]. The *Ipomoea* genus has been studies for their phytochemicals from mid of 20th century. Some species of *Ipomoea* reported for their antimicrobial, analgesic, anticancer, spasmogenic, hypertensive and psychotomimetic activities. The common photochemical constituents that are ergoline alkaloids, nortropane alkaloids, glycolipids, coumarins, isocoumarin, phenolics compounds, norisoprenoids, diterpene, benzenoids, flavonoids, antocianosides, lignin and triterpenes has been identified as biological active constituents.

However, *Ipomoea campanulata* species is not well exploited for their phytochemical studies as well as biological activities [3].

The objective of this study is to phytochemically analyze *Ipomoea campanulata*, determine total antioxidant activity using 2-2 diphenyl-1-picrylhydrazyl (DPPH) & Nitric oxide radical scavenging, and characterize it through Liquid Chromatography- High Resolution Mass Spectroscopy (LC-HRMS). It is also includes cytotoxic properties of *I. campanulata* using HepG2 and MCF7 cell lines.

The general information regarding plant under study is as follows:

Classification: Kingdom – Plantae, Phylum – Tracheophyta, Class - Magnoliopsida Order – Solanales, Family – Convolvulaceae, Genus – *Ipomoea,* Species – *campanulata* L. viz *illustris* (C. B. Cl.) Prain

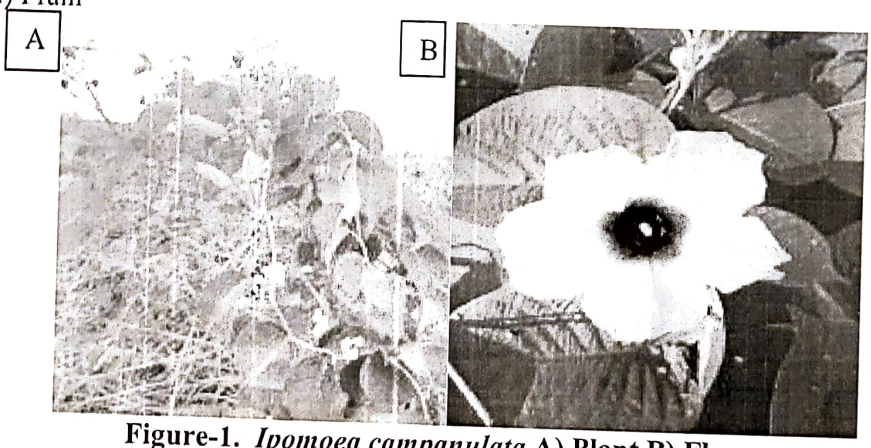

Figure-1. *Ipomoea campanulata* A) Plant B) Flower.

Ipomoea campanulata- Stout climbers with 8 x 9 cm leaves which are broadly ovate to orbicular, petiole to 8 cm long. Flowers are 1 or 2 together containing pedicels 3.5 cm long and stout. The sepals are 1 x 1 cm in size, orbicular in shape, outer 3 pubescent outside, growing in fruit; filaments hairy at base. Seeds are black and hairy on angles (**Figure-1**).

2. EXPERIMENTAL SECTION

2.1 Preparation of extract

The leaves of *Ipomoea campanulata* were collected from western ghat region covered under Pune, Maharashtra, India. Location lies in19°19'01.4"N 73°57'11.3"E. Dust particles were removed by washing leaves with tap water followed by distilled water. The leaves then shed dried for 15 days at room temperature and powdered using mechanical grinder. The crude extract was obtained by mixing 10 g of powder in 100 ml solvent keeping mixture overnight followed by filtration with Whatman No.1 filter paper. Crude extract concentrated by evaporating maximum solvent using rotavapor. Concentrated extract then taken on petriplates & evaporated completely. After obtaining yield, extract was re-dissolved in solvent for further use.

2.2 Phytochemical screening of extract:

The qualitative phytochemical tests were performed for Alkaloids, Anthraquinones, Proteins& amino acids, Saponins, Flavonoids, Phenols, Terpenoids, Sterols, Tannins and Glycosides to know their existence in plant extracts [4-6].

2.2.1 Test for alkaloids

One ml of plant extract was mixed with 1 ml Mayer's reagent. Yellow precipitation indicates the presence of alkaloids.

2.2.2 Test for anthraquinone

The 0.5 ml of plant extract added in 1 ml of 10 % ammonium solution, shaken vigorously for 1 min. Red color formation indicates the presence of anthraquinone.

2.2.3 Test for proteins and amino acids

Ninhydrin test – few drops of Ninhydrin reagent added in 1 ml plant extract, kept in boiling water bath for 30 sec to 1 min. Purple color formation indicates the presence of proteins and amino acids.

2.2.4 Test for Saponin

Foam test –
The 1 ml of plant extract shaken continuously for 15 min. Formation of layer of foam indicates the presence of saponin.

2.2.5 Test for flavonoids

H_2SO_4 test- few drops of conc. H_2SO_4 added in 1 ml of plant extract. Orange color formation indicates the presence of flavonoids.
Aluminium chloride test –In the adequate amount of plant extract equal amount of 1% aluminium chloride solution was added. The formation of light-yellow color indicates the presence of flavonoid.

2.2.6 Test for phenols

Ferric chloride test – 1 ml of plant extract mixed with 2 ml of 5% or 1 N aqueous ferric chloride. Formation of deep blue or black color indicates the presence of phenols.

2.2.7 Test for terpenoids

H2SO4 Test- 1 ml of plant extract mixed with 0.4 ml of chloroform, evaporate on boiling water bath, cool and added 0.6 ml conc. H_2SO_4, the solution was boiled for 30 sec. Formation of gray color indicates the presence of terpenoids.
Salkowski's test – In a solution of 1 ml plant extract and 2ml of chloroform, 3 ml of conc. H2SO4 added from the side of the test tube. The reddish-brown coloration at the interface indicates the presence of terpenoids.

2.2.8 Test for sterols

Salkowski's test – In a mixture of 1 ml plant extract and 2ml of chloroform, 1ml of conc. H2SO4 added from the side of the test tube. The chloroform layer appears red and the acid layer appears greenish yellow fluorescent this indicates the presence of sterols.

2.2.9 Test for tannins

Ferric chloride test –1 ml of plant extract diluted with distilled water in 1:1 proportion them 2 – 4 drops of 1 N ferric chloride solution was added. Black color formation indicates the presence of tannins.

2.2.10 Test for glycosides

Leibermann's test – In a mixture of 1 ml of acetic acid and 1 ml of chloroform, 1 ml of plant extract was added. Then conc. H_2SO_4 was added drop wise. The green color formation indicates the presence of glycosides.
Salkowski's test- 2 ml of conc. H_2SO_4 was added drop wise to the plant extract. The reddish-brown color formation indicates the presence of glycosides.

2.3 Total Phenolic Content

The total phenolic content in extracts was determined by Folin- Ciocalteu method [7]. Total phenolic content in extract presented as milligram Gallic Acid Equivalent (GAE).

2.4 Total Flavonoid Content

Total flavonoid content in plant extracts were estimated by method described by Khlif et al. [5]. Total Flavonoid content in extracts presented as milligram Quercetin equivalent.

2.5 Total Protein Content by lowry's method

The total protein content was evaluated by Lowry et al. [8] using BSA as a standard. The total protein content was expressed in mg/ml of extract.

2.6 Total Reducing Sugar Content by DNSA method

Determination of total reducing sugar content was done by DNSA method using glucose as a standard. The total reducing sugar content expressed in mg/ml of extract.

2.7 Antioxidant activity

2.7.1 DPPH radical scavenging activity

The DPPH free radical scavenging activity of extract was determined using methods described by Nguemfo et al. [9]. Briefly, 1ml of 1 mg/ml extract/Ascorbic acid added to 3 ml of a DPPH solution (0.004 % in methanol). Then mixtures were incubated in dark for 30 min. After incubation absorbance was recorded at 517 nm in a spectrophotometer using solvent as blank. The percentage inhibition of the DPPH radical calculated for extract and Ascorbic acid according to formula:

$\%\ \text{Inhibition} = [A_c - A_t]\ A_c \times 100$

Where, 'A_c' is the absorbance of the DPPH control at $t = 0$ min and 'A_t' is the absorbance in presence of Extract/Ascorbic acid at a particular concentration at t = 30 min.

2.7.2 Nitric oxide radical scavenging activity

The Nitric oxide radical scavenging activity was evaluated by the method describe by Gangwar et. al., (2014) [10]. 1mg/ml of extract/Ascorbic acid added in 3 ml of 10 mM Sodium Nitroprusside (prepared in 0.5 M phosphate buffer (pH 7.4)). Mixtures were incubated for 150 min at 25^{0}C then1.0 mlGriess reagent [(0.33% sulfanilicacid in 20% glacial acetic acid)+ 1 ml of naphthylethylenediamine dichloride (0.1% w/v)] was added. Spectrophotometrically absorbance was measured at 546 nm. Thenitric oxide radical inhibition was calculatedusing followingformula:

% inhibition of NO radical= $(A_0 - A_t) / A_0$ X 100

Where, A_0 is the absorbance before reaction and A_t is the absorbance after reaction.

2.8 Detection of phytochemicals by LC-HRMS

LC-HRMS/MS phytochemical profiling was performed on an Agilent6540 UHDUHPLC system, diode array detector and electrospray ionization-quadrupole-time of flight mass spectrometer (ESI-QTOF-MS). An Agilent zorbax SB-C18 (150 X 0.5 mm, 5µ) column was used. The following gradient use: 0.1% formic acid in water (A), 0.1% formic acid in methanol (B). Injection volume was 10 µl, flow-rate was 0.5 ml/min. ESI-Q-TOF-MS analysis was done in positive ionization mode with mass range 100-1700 m/z [11-12].

2.9 Cytotoxicity capacity by MTT assay

Cytotoxicity of *I. campanulata* plant extracts was evaluated by using the method described by Abubakar et al. [13] and Ihmaid et al. [14] with modification. Cells with the concentration of 1 × 10^{4}cells/ml incubated in culture medium for 24 h at37°C with 5% CO2. In 100µl culture medium, cells were seeded as1 x10^{4}cells/well with the sample (100µl/ml) concentration 10, 40, 100 µg/ml. DMSO (0.2% in PBS) & cell line incubated in wells as control. To know the control cell survival and percentage of live cells after culture controls were maintained. The incubation of cell cultures was done for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientificBB150). Medium was completely removed after incubation, 20 µl of MTT reagent (5mg/min PBS) was added and cells were incubated for 4 hrs at 37^{0}C in CO2incubator. Formation of formazan crystals in the wells was observed under microscope. Viable cells reduced yellowish MTT into dark colored formazan. After incubation medium was removed completely and 200µlof DMSO added. Then kept for 10 min and again incubated at 37^{0}C (wrapped with aluminium foil). Absorbance of each sample was measured by a microplate reader (Benesphera E21) at a wavelength of 550 nm. The percent inhibition was calculated by using formula:

% Inhibition = [A(control) – A (extract/Standard)/ A(control)]* 100

3. RESULTS & DISCUSSION

The extraction of *Ipomoea campanulata* leaves were done by methanol (IC-Me) and ethyl acetate (IC-EA) solvents. For to know the presence of primary metabolites such as amino acids, carbohydrates, proteins, and secondary metabolites such as phenolics, flavonoids, saponins, tannins, terpenoids, glycosides, and anthraquinones etc. qualitative phytochemical tests were performed. **Table-1** shows the results of the qualitative phytochemical analysis.

Table-1. Qualitative phytochemical analysis of *I. campanulata* leaves extract. (+) Present, (-) Absent.

Constituents	Test	*I.campanulata*	
		IC-Me	IC-EA
Alkaloids	Test for alkaloids	+	+
Anthraquinones	Test for anthraquinone	-	-
Proteins and amino acids	Ninhydrin test	+	+
Saponins	Foam test	+	+
Flavonoids	i)H2SO4 test	+	+
	ii)Aluminum Chloride test	+	+
Phenols	Ferric chloride test	+	+
Terpenoids	i)H2SO4 test	+	+
	ii)Salkowski's test	+	+
Sterols	Test for sterol	+	+
Tannins	Ferric chloride test	+	+
Glycosides	i) Leibermann's test	+	+
	ii)Salkowski's test	+	+

The phytochemicals like phenolic, flavonoids, proteins and reducing sugars were analysed quantitatively. **Table-2** shows the results of the quantitative phytochemical analysis.
The antioxidant activityof *I. campanulata* leaves extracts were evaluated using DPPH & Nitric oxide free radical scavenging method.Ascorbic acid was used as a standard. **Table-3** represents the results of in-vitro Antioxidant activity.

Table-2. Analysis of total content of Phenolic, Flavonoid, Protein & Reducing sugar in *Ipomoea campanulata* leaves extract. Results were presented as Mean ± SD.

Sample (1 mg/ml)	*I. campanulata*	
	IC-Me	IC-EA
Total Phenolic content (Gallic acid equivalent inmg/ml)	0.440± 0.09	0.380± 0.08
Total Flavonoid content (Quercetin equivalent in mg/ml)	0.071± 0.002	0.057± 0.002
Total Protein content	0.013± 0.004	0.022± 0.006
Total Reducing sugar content	0.077± 0.009	0.092± 0.015

Table-3. Antioxidant activity of *Ipomoea campanulata*plant leaves extract. Results were presented as Mean ± SD.

	Antioxidant	
Sample 1mg/ml	DPPH Free Radical scavenging (% inhibition)	NO radical Scavanging assay (% inhibition)
Standard (Ascorbic acid)	74.46±1.62	78.12± 2.55
IC-Me	68.75±1.08	56.25±5.10
IC-EA	67.15±0.76	60.93±6.93

The phytochemicals detected from *I. campanulata* methanolic leaf extract by using the LC-HRMS/MS method are presented in **Table-4**. The greater number of compounds detected from phenolic, flavonoid, terpenes and terpenoids group.

Cytotoxicity analysis was done by MTT assay. The results of MTT assay are represented in **Table-5**.

The methanolic extract of *I. campanulata* showed a significant inhibition for HepG2 and MCF7 cells.

Table-4. List of bioactive components detected by LC-HRMS/MS in methanolic extract of *I. campanulata.*

Sr.No	Name of compound	RT	Mass	M/Z	Molecular Formula
1	Sebacic acid	0.49	202.1207	202.1444	C10 H18 O4
2	Pirbuterol	0.64	240.1477	241.1549	C12 H20 N2 O3
3	Trolamine	0.66	149.1058	132.1025	C6 H15 N O3
4	Brusatol	6.52	520.1952	503.192	C26 H32 O11
5	Myricitrin	6.57	464.0956	465.1032	C21 H20 O12
6	Quercetin	7.05	550.0964	551.1039	C24 H22 O15
7	(±)-Taxifolin	7.2	304.0586	287.0554	C15 H12 O7
8	Hymenoxon	8.11	282.1466	283.1539	C15 H22 O5
9	Zingerone	9.78	194.095	195.1024	C11 H14 O3
10	Sarcostin	1025	382.2358	365.2326	C21 H34 O6
11	Ricinoleic acid	11.36	298.2511	298.2744	C18 H34 O3
12	Populin	11.68	390.1318	373.1284	C20 H22 O8
13	Sphinganine	11.7	303.1473	302.3059	C18 H39 N O2
14	Heliotrine	11.75	313.1891	296.1857	C16 H27 N O5
15	Embelin	12.85	294.1834	277.1801	C17 H26 O4
16	Phytol	14.06	296.3082	314.3421	C20 H40 O
17	Graphinone	14.99	296.1624	279.1592	C16 H24 O5
18	Misoprostol	17.12	382.272	365.2695	C22 H38 O5
19	Lutein	20.53	568.4285	551.425	C40 H56 O2
20	Typhasterol	23.58	448.3561	431.3529	C28 H48 O4

Table-5. Results of cytotoxic activity on HepG2 and MCF7 cell lines with methanol extract of *I. campanulata*.

MTT assay					
Sample	Concentration (µg/ml)	Mean absorbance		HepG2 cell lines (% inhibition)	MCF7 cell lines (% inhibition)
		HepG2	MCF7		
	Control	0.973	1.538		
5FU (Std)	10	0.233	0.731	76.05	52.47
	40	0.214	0.673	78.00	56.24
	100	0.156	0.633	83.96	58.84
IC-Me	10	0.773	0.653	20.55	57.54
	40	0.657	0.589	32.47	61.70
	100	0.425	0.523	56.32	65.99

4. CONCLUSION

The present study revealed that, *I. campanulata* has a wide range of secondary metabolites with the higher phenolics and flavonoid content. The *I. campanulata* plant extracts showed the remarkable Antioxidant and cytotoxic activities. The confirmation of bioactive components in *I. campanulata* was done by LC-HRMS profile. Hence, *I. campanulata* can be act as a good source of the bioactive components. Exploitation and further studies of *I. campanulata* plant may help in identifying and purifying crude drug for medicinal use.

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