

Ipomoea reniformis: Isolation, Characterization, and Evaluation of Scopoletin and its Antioxidant and Cytotoxic Activities

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Abstract: The *Ipomoea reniformis* is found predominantly all over India and is used traditionally for several diseases including for the cure of cancer. This study aimed to isolate and characterize phytoconstituents from bioactive extract, evaluate antioxidant and cytotoxic properties of chloroform (CH), ethyl acetate (EA), ethanol (ET), hydroalcoholic (HA) (50%v/v) extracts and isolated compound. Antioxidant property was evaluated by using 1,1-Diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging assay and cytotoxic potential was evaluated by using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) Assay. All the extracts (CH, EA, ET, HA) of *Ipomoea reniformis* exhibited significant antioxidant potential but the highest antioxidant activity (94.84 ± 1.56 % at 1000 µg/ml) was shown by ET extract with IC₅₀ value of 52.24 µg/ml and SM (scopoletin) isolated from ET extract showed more potent antioxidant activity IC₅₀ value of 52.24 ± 0.9367 µg/ml, and the highest antioxidant activity (96.29 ± 0.86 %) at 1000 µg/ml compared to ET extract. The percent cell viability of MCF-7, A-549, and HCT-116 human cell lines was evaluated by treatment with CH, EA, ET, HA extracts and SM. The percent cell viability was found to be, ET (4.4117 ± 6.2711%, 0.0605 ± 0.7748%, 9.8290 ± 2.1149%) and SM (4.23 ± 1.38%, 0.059 ± 0.32 %, 8.92 ± 0.87%) for MCF-7, A-549, HCT-116 respectively. Therefore, from the present study it was concluded that isolated SM has more potent antioxidant and cytotoxic potential than ET extract of *Ipomoea reniformis*.

Keywords: Scopoletin, *Ipomoea*, Antioxidant, Cytotoxic, DPPH.

1. INTRODUCTION

The Rigveda, the oldest text in India, contains a list of medicinal plants with curative properties which showed the way to explore the plants for their use in the treatment of various diseases [1],[2]. By using the knowledge of traditional medicine various discoveries have come up with newer lead molecules and contributed a lot to the development of medicine [3],[4].

As per World Health Organization (WHO), each year more than 10 million people are getting cancer [5]. It is known to be the second cause of mortality throughout the globe [6], contributing 20% of all deaths [7],[8]. The drugs obtained from the plants play a crucial role in drug discovery [9]. Some of the natural products obtained from the plants that are now being used as anticancer are Vincristine, Vinblastine [10], Etoposide, Teniposide (Podophyllotoxin derivatives) [11],[12],[13], Paclitaxel, Docetaxel (Taxol derivative) [14],[15], Camptothecin, Homoharringtonine, Elliptinium, etc. [16]. Currently, the available drugs possess undesirable and untoward effects. Therefore, needs to search for a newer moiety that has anticancer potential with minimum side effects.

The plant *Ipomoea reniformis* chois belongs to the Convolvulaceae family and is a creeper herb. It is commonly known as Udirkana and Mushakparina as the shape of the leaves is like a rat ear. It is distributed throughout India [17]. It has different chemical constituents reported till date such as scopoletin, caffeic acid, pcoumaric acid, ferulic acid and tannins.

In various traditional systems of medicine, it is being used to treat various diseases such as neuralgia, wounds, fever, diabetes, rheumatism, skin disorders, and eye infections [18],[19].

The literature survey revealed that plant has been reported to have anti-urolithiasis effect [20], antimicrobial activity

[21], [22], Antiepileptic [23], hepatoprotective activity [24], anticancer activity [25],[26], hypotensive, diuretic and angiotensin-converting enzyme (ACE) inhibitory activities [27], Antitumor activity and antioxidant activity [28], hypoglycemic effects [29], Antiulcer and antioxidant activity [30].

1.1 Objectives

The present study aimed to extract the plant material by using various solvents, isolate and characterize phytoconstituents from bioactive extract, and evaluate the antioxidant and cytotoxic properties of chloroform (CH), ethyl acetate (EA), ethanol (ET), hydroalcoholic (HA) (50%v/v) extracts, and isolated compound.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The aerial part of *Ipomoea reniformis* was collected in September from the Sirocha forest division, Gadchiroli district, Maharashtra. The dried plant specimen was identified and authenticated at the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The voucher plant specimen (10320) has been deposited for future reference.

2.2 Chemicals, Extraction and Isolation

All the solvents used for extraction and isolation were of Analytical Reagent grade. 1,1-Diphenyl 2-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals, USA. The adsorbent used for column chromatography was silica gel-60-120 (Merk).

2.3 Procedure for Extraction and Isolation

The aerial part of *Ipomoea reniformis* was first defatted with petroleum ether and was further successively extracted by using soxhlet apparatus with various solvents such as chloroform, ethyl acetate, and ethanol. Hydroalcoholic (50%v/v) extract was prepared by maceration process. The bioactive ethanolic extract was chromatographed on silica gel column chromatography. The column was first eluted with hexane and the polarity was gradually increased by ethyl acetate (100:0, 95:5, 90:10, 85:15, 80:20v/v). A total of 109 fractions of 50ml each were collected, the fractions were pooled and collected by performing the TLC. The fraction no. 15 to 22 were collected and it was further purified by using re-crystallization to get a pure compound (SM) [31].

2.4 Phytochemical Screening

The different extracts obtained such as chloroform (CH), ethyl acetate (EA), ethanol (ET) and hydroalcoholic (HA) (50%v/v) extracts were screened for the presence of various secondary metabolites such as coumarins, alkaloids, glycosides, terpenes, tannins etc. by following the standard procedure [32].

2.5 DPPH Radical Scavenging Assay

The determination of DPPH radical scavenging assay was performed as per the method Oyaizu (1986) [33].

2.6 MTT Assay

Cytotoxicity of the CH, EA, ET, HA and SM to MCF-7, A549, and HCT 116 cell lines was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO₂. Next day cells were treated from 1-1000 µg/ml of the formulations (different concentrations were prepared in incomplete medium). After incubation for 24 hours, MTT Solution (a final concentration of 250µg/ml) was added to cell culture and further incubated for 2 h. At the end

of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 μ l Dimethyl Sulfoxide (DMSO) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm [34].

2.7 Analytical Methods

Thin Layer Chromatography was performed on silica gel GF 254 precoated (Merck) plates. Infrared spectrum was recorded with FTIR (Shimadzu), ^1H , and ^{13}C spectra recorded on Bruker (400MHz and 100MHz) in DMSO using TMS as an internal standard. ESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA).

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening

It revealed the presence of alkaloids, glycosides, coumarins, steroids, saponins, flavonoids in chloroform, ethyl acetate and ethanol extracts.

3.2 Characterization of isolated compound (SM)

It was a white colour powder. The mass spectrum of SM showed m/z 192.1 which corresponds to molecular formula $\text{C}_{10}\text{H}_8\text{O}_4$. **FTIR**

A peak at 3337.11 corresponds to the phenolic OH group. A peak at 2925.13 shows due to C-H stretching. A peak at 1703.99 corresponds to a carbonyl group.

^1H NMR (400MHz, DMSO, δ , TMS=0): δ 3.799 ppm (3H, S), δ 6.106 ppm (1H, d, $J=2.0$ Hz, H-3), δ 7.726 ppm (1H, d, $J=2.0$ Hz, H-4), δ 7.009 ppm (1H, d, $J=1.6$ Hz, H-5), δ 6.718 ppm (1H, d, $J=1.6$ Hz, H-8).

^{13}C NMR (100MHz, DMSO, δ , TMS=0): δ 161.15 (C-1), δ 110.84 (C- 2), δ 144.45 (C-3), δ 111.95 (C-4), δ 109.41 (C- 5), δ 145.56 (C-6), δ 150.00 (C-7), δ 103.21 (C-8), δ 151.52 (C- 9), δ 56.31 (C-10).

^1H NMR spectrum showed singlet at δ 3.799 ppm corresponding to three protons of the methoxy group. The two doublets at δ 6.106 ppm and δ 7.726 ppm with a coupling constant of 2.0 Hz correspond to H-3 and H-4 respectively. The two doublets at δ 7.009 ppm and δ 6.718 ppm with a coupling constant of 1.6 Hz correspond to aromatic protons.

The ^{13}C -NMR spectrum of SM showed 10 carbon resonances. The carbonyl group appeared at 161.15 ppm and the methoxy group at 56.31 ppm. The oxygenated aromatic carbons appeared at 144.45, 145.56, 150.00, and 151.52 ppm, and the non-oxygenated aromatic carbons were detected at 103.21, 109.41, 110.84, and 111.95 ppm.

The comparison of all the spectral data with the literature available led us to conclude the structure is scopoletin.

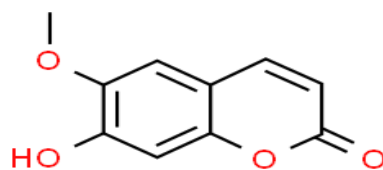


Figure 1. Structure of Scopoletin

3.3 DPPH Radical Scavenging Assay

All the extracts of *Ipomoea reniformis* have significantly reduced the DPPH radicals in a concentration dependent manner. After the study, it was found that ethanolic extract showed potent antioxidant activity with IC₅₀ value of 52.24 µg/ml, and the highest antioxidant activity i.e., 94.84 ± 1.56 % at 1000 µg/ml as compared with other extracts. IC₅₀ value of chloroform, ethyl acetate and hydroalcoholic extract was found to be 595µg/ml, 91.92 µg/ml, and 57.89 µg/ml respectively as shown in figure 1.

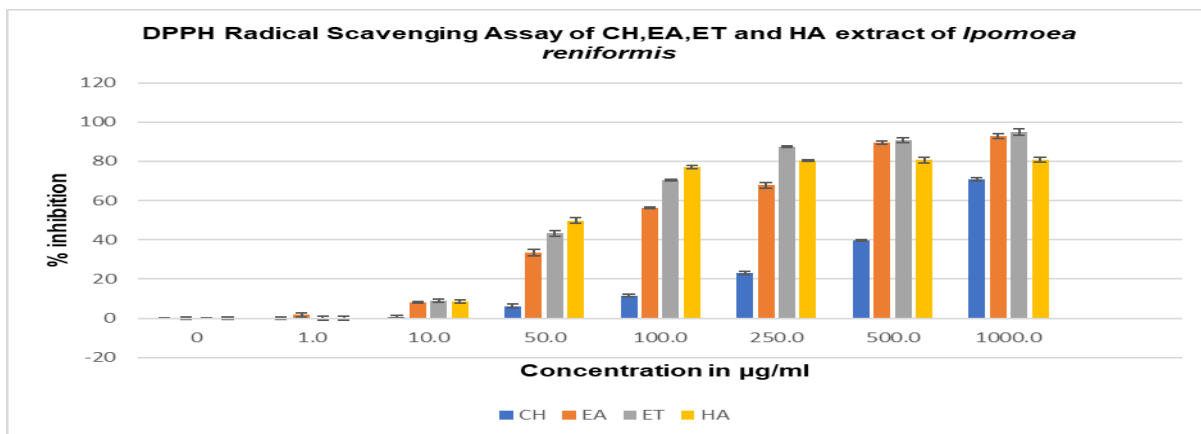


Figure 2. Percent inhibition concentration (50%) of various extracts when treated with DPPH reagent

The SM obtained from *Ipomoea reniformis* have reduced the DPPH radicals in a concentration dependent manner as shown in figure 2.

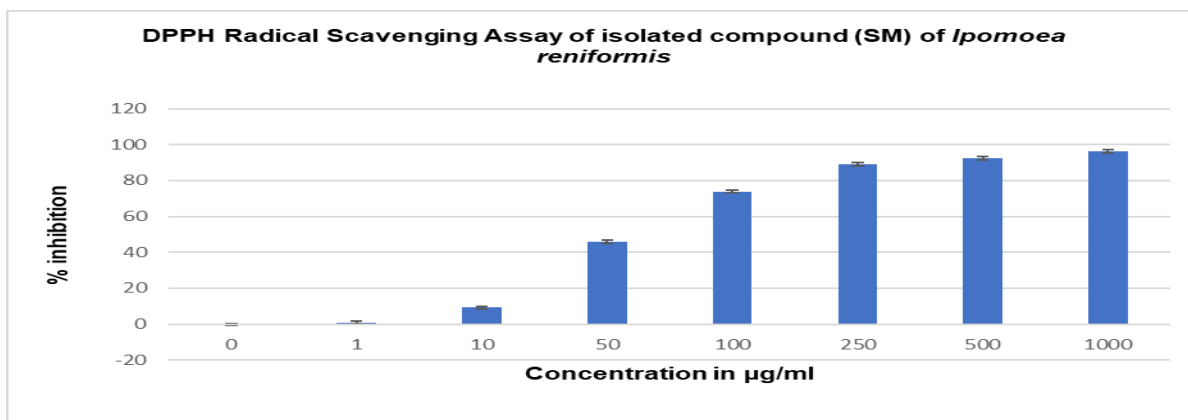


Figure 3. Percent inhibition concentration (50%) of isolated compound (SM) when treated with DPPH reagent

It was found that SM obtained from ethanolic extract revealed potent antioxidant activity with IC_{50} value of $52.24 \pm 0.9367 \mu\text{g/ml}$, and the highest antioxidant activity i.e., $96.29 \pm 0.86 \%$ at $1000 \mu\text{g/ml}$ was shown in SM which is more than the ethanolic extract from which it was isolated. The antioxidant effect is essential to maintain the homeostasis of the system in cancer therapy and thereby ability of the immune system to fight against tumour antigens which prevents the angiogenesis of cancer cells. The presence of flavonoids, tannins, coumarins in the ET extract of *Ipomoea reniformis* could be responsible for the antioxidant effect [35].

3.4 Evaluation of Cytotoxic Activity

3.4.1. MCF-7 Cell Viability Assay by MTT

MCF-7 cell viability was determined in the presence of increasing concentrations of plant extracts of *Ipomoea reniformis* as shown in Table 1.

Table 1. Cell Viability (%) of MCF-7 cell lines for CH, EA, ET, HA extracts and SM

Concentration $\mu\text{g/ml}$	Cell Viability (%)				
	CH	EA	ET	HA	SM
0	100 \pm 2.5829	100 \pm 10.1227	100 \pm 5.1256	100 \pm 6.8641	100 \pm 1.76
1	96.3068 \pm 1.7589	82.7778 \pm 4.6591	97.5490 \pm 5.3921	93.0355 \pm 6.1164	96.42 \pm 1.81
10	78.9773 \pm 2.7249	62.7778 \pm 6.7510	93.1372 \pm 5.2491	91.9757 \pm 2.6631	87.17 \pm 2.46
50	60.5114 \pm 4.6478	44.4444 \pm 8.6543	86.2745 \pm 10.030	85.6169 \pm 5.6427	74.36 \pm 1.21
100	53.6932 \pm 1.8772	37.7778 \pm 10.122	67.1568 \pm 12.436	81.7562 \pm 1.9817	58.12 \pm 1.55
250	46.5909 \pm 5.0073	33.3333 \pm 3.2710	35.7843 \pm 3.4313	74.6404 \pm 2.9490	29.18 \pm 1.42
500	24.1477 \pm 3.1592	32.2222 \pm 7.4259	6.3725 \pm 3.7864	64.1937 \pm 1.9359	5.89 \pm 1.43
1000	13.3523 \pm 10.542	25 \pm 7.3917	4.4117 \pm 6.2711	48.5238 \pm 3.1809	4.23 \pm 1.38

The values presented are mean \pm standard error mean, $n = 4$

Table 1 depicts the percent viability of MCF-7 cells after incubation for 24 hours with Chloroform, Ethyl acetate, Ethanol and hydroalcoholic extracts at different concentrations. MCF-7 cells which were maintained in DMEM without any plant extract have a percent viability 100%. The percent viability of MCF-7 cells was reduced in a significant, inversely in dose response manner due to treatment with Ethanol extract. The highest activity was observed at $1000 \mu\text{g/ml}$ concentration of ethanol extract, the % viability at this concentration found to be observed was $4.4117 \pm 6.2711\%$. The IC_{50} value of Chloroform, Ethyl acetate, Ethanol and Hydroalcoholic extracts was $126.5 \pm 0.8982 \mu\text{g/ml}$, $58.7 \pm 0.237 \mu\text{g/ml}$, $146.4 \pm 0.9445 \mu\text{g/ml}$ and $804.7 \pm 0.8447 \mu\text{g/ml}$ respectively.

The percent viability of MCF-7 cells was reduced in a significant, inversely in dose response manner due to treatment with isolated compound (SM). Highest activity was observed at $1000 \mu\text{g/ml}$ concentration, the % viability at this concentration found to be observed was $4.23 \pm 1.38\%$. The IC_{50} value of isolated compound (SM) was $103.12 \pm 0.9234 \mu\text{g/ml}$.

3.4.2. A-549 Cell Viability Assay by MTT

The percentage viability of human lung adenocarcinoma epithelial cell line A-549 was determined in the presence of increasing concentrations of CH, EA, ET, HA extracts and SM as shown in Table 2.

Table 2. Cell Viability (%) of A-549 cell lines for CH, EA, ET, HA extracts and SM

Concentration $\mu\text{g/ml}$	Cell Viability (%)
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	CH	EA	ET	HA	SM
0	100 ± 5.0635	100 ± 4.3535	100 ± 1.1966	100 ± 1.7135	100 ± 1.72
1	81.5361± 0.796	89.3203 ± 7.509	91.3385 ± 1.142	93.6591 ± 2.110	87.38 ± 1.81
10	79.7578 ± 0.930	83.4951 ± 8.233	72.8043 ± 1.180	88.4235± 2.479	70.20 ± 1.44
50	78.4714 ± 3.484	75.5894 ± 5.229	49.2428 ± 2.623	70.4479 ± 3.289	46.18 ± 2.27
100	74.8013 ± 2.414	71.0587 ± 4.874	33.2525 ± 1.275	46.1314 ± 5.222	32.12 ± 1.31
250	69.2395 ± 1.728	57.2353 ± 1.560	9.2065 ± 1.0278	34.2641 ± 3.629	9.02 ± 1.12
500	49.4892 ± 0.293	47.3416 ± 1.080	1.1508 ± 0.6284	9.4240 ± 0,9547	1.06 ± 0.58
1000	37.2304 ± 2.834	36.2359 ± 2.722	0.0605 ± 0.7748	3.5485 ± 0.5235	0.059 ± 0.32

The values presented are mean ± standard error mean, $n = 4$

In normal conditions, without any extract treatment, the percent viability of cells was found to be 100%. The IC_{50} value of CH, EA, ET and HA extract for A-549 cell lines was $463.1 \pm 0.4856 \mu\text{g/ml}$, $96.48 \pm 0.9775 \mu\text{g/ml}$, 39.24 ± 0.9732 and $357.4 \pm 0.724 \mu\text{g/ml}$ respectively. The percent viability of A-549 cells was reduced in a significant, inversely in dose response manner due to treatment with Ethanol extract. Highest activity was observed at 1000 $\mu\text{g/ml}$ concentration of ethanol extract, the % viability at this concentration found to be observed was $0.0605 \pm 0.7748\%$.

The percent viability of A549 cells was reduced in a significant, inversely in dose response manner due to treatment with isolated compound (SM). Highest activity was observed at 1000 $\mu\text{g/ml}$ concentration, the % viability at this concentration found to be observed was $0.059 \pm 0.32 \%$. The IC_{50} value of isolated compound (SM) was $36.89 \pm 0.8923 \mu\text{g/ml}$.

3.4.3. HCT-116 Cell Viability Assay by MTT

HCT-116 cell viability was determined in the presence of increasing concentrations of plant extracts of *Ipomoea reniformis* as shown in Table 3.

Table 3. Cell Viability (in %) of HCT-116 cell lines for CH, EA, ET, HA extracts and SM

Concentration $\mu\text{g/ml}$	Cell Viability (%)				
	CH	EA	ET	HA	SM
0	100 ± 8.4938	100 ± 2.2827	100 ± 3.1863	100 ± 4.2224	100 ± 1.87
1	83.1363 ± 5.210	73.8538± 4.761	79.3706 ± 6.362	85.270 7 ± 7.315	76.13 ± 1.4
10	62.2272 ± 7.323	57.8756 ± 1.697	63.3644 ± 3.647	69.267 5 ± 7.820	61.12 ± 1.19
50	55 ± 2.7624	48.2523 ± 1.280	58.1973 ± 4.284	65.207 0 ± 5.992	57.67 ± 1.28
100	48.1818 ± 1.777	45.7557 ± 0.770	47.1251 ± 5.325	56.449 0 ± 1.813	45.22 ± 1.05
250	42.0909± 2.028	36.5410 ± 0.816	43.5508 ± 2.426	49.761 1 ± 2.218	40.81 ± 1.45
500	34.5± 0.9801	30.867 ± 1.2688	22.9215 ± 2.379	34.116 2 ± 1.868	19.71 ± 0.98
1000	21.1363±	21.7884 ±	9.8290 ±	28.941	8.92 ±
	2.694	1.735	2.1149	0 ± 1.470	0.87

The values presented are mean ± standard error mean, $n = 4$

It depicts the percent viability of HCT-116 cells after incubation for 24 hours with Chloroform, Ethyl acetate, Ethanol, and hydroalcoholic extracts at different concentrations. HCT-116 cells which were maintained in DMEM without any plant extract have a percent viability of 100%. The percent viability of HCT-116 cells was reduced in a significant, inversely in dose-response manner due to treatment with Ethanol extract. The highest activity was observed at 1000 µg/ml concentration of ethanol extract, the % viability at this concentration found to be observed was $9.8290 \pm 2.1149\%$. The IC₅₀ value of Chloroform, Ethyl acetate, Ethanol, and Hydroalcoholic extracts was 68.84 ± 0.9649 µg/ml, 35.1 ± 0.9776 , 55.34 ± 0.8892 µg/ml and 149.5 ± 0.9535 µg/ml respectively.

The percent viability of HCT-116 cells was reduced in a significant, inversely in dose-response manner due to treatment with an isolated compound (SM). The highest activity was observed at 1000 µg/ml concentration, the % viability at this concentration found to be observed was $8.92 \pm 0.87\%$. The IC₅₀ value of the isolated compound (SM) was 52.19 ± 0.7213 µg/ml.

CONCLUSIONS

In the present work, bioactivity-guided isolation was performed from ET extract, one single compound (SM) was isolated and which was found to be scopoletin. The antioxidant and cytotoxic potential of CH, EA, ET, HA extracts and SM were studied by using DPPH scavenging assay and MTT assay respectively. From this study, it was concluded that ET extract showed significant antioxidant and cytotoxic activity but the isolated compound (SM) revealed the highest antioxidant and cytotoxic activity than ET extract. In the future, there is further scope for the isolation of other compounds such as flavonoids, tannins, and steroids which are also responsible for their antioxidant and cytotoxic properties. Further molecular modifications may give the potent anticancer drug to the human mankind.

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