Journal of Chemical Health Risks

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JCHR (2025) 15(1), 245-254 | ISSN:2251-6727

Investigation of Phytochemical Potential of Various Extracts of Ipomoea *Reniformis Chois*

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(Received: 16 November 2024 Revised: 20 December 2024 Accepted: 04 January 2025)

KEYWORDS

Soxhlet apparatus, A cid neutralizing capacity, Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry (MS), and Fourier Transform Infrared (FTIR)

ABSTRACT:

This study aimed to provide pharmacological evidence of chloroform (CE), Ethyl Acetate (EA), Ethanol (EE) and Hydroalcoholic (HE) extract of *Ipomoea Reniformis Chois* (Convolvulaceae) preventing or healing peptic ulcers. The shade dried leaves of *Ipomoea reniformis* was extracted using Soxhlet apparatus. The extracts were subjected to determination of physical constants and phytochemical tests, Acid neutralizing capacity and Antioxidant capacity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and Superoxide Radical Scavenging Activity. The extracts were characterization by thin layer chromatograms. Isolated compound were analyzed by UV, IR, NMR and Mass spectroscopy. The results showed total solid content 98.85 ±0.04 which is highest in physical constant, the percent yield was found 11.06 % highest for ethanol extract. All four extracts showed the presence of Glycosides, Flavonoids and Carbohydrates in abundance. Total phenolic content was highest 28.12 ±1.08 for EE extract while 6.21 ±0.13, 21.40 ±0.32, 16.84 ±0.19 CE, EA and HE presented unit milligrams of Gallic acid equivalent respectively. Total Flavonoids content was highest in EA 59.29 ±1.87 and EE 59.09 ±1.87 while CE has 52.32 ±0.69 and HE 24.54 ±1.87 in mg of RU/g of extract equivalent. The bioactive compounds identified in *Ipomoea reniformis* were correlated with their potential role in antiulcer activity based on the in vitro studies. To further confirm the identity of the active compounds, advanced techniques such as Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry (MS), and Fourier Transform Infrared (FTIR) spectroscopy were employed. These techniques allow for the structural elucidation of isolated bioactive compounds.

1. Introduction

Herbal medicine has a long history in treatment of several diseases. Medicinal herbs are used to treat illness, maintain and promote health available as inexpensive source of primary health care, especially in the absence of access to modern medical facilities[1]. Peptic Ulcers are characterized histopathological as break in the mucosa of the alimentary tract that reaches out through the muscularis mucosae into the submucosa or more profound. Symptoms of a peptic ulcer include nausea, vomiting, bloating, appetite changes, and burning. Peptic ulcers aetiology may orient from pathophysiologic abnormalities, environmental or hereditary factors and may be due microbial indulgence like Helicobacter pylori (H. Pylori) or may be drug induced like nonsteroidal anti-inflammatory (NSAIDs), or other factors that affect mucosal defense and healing systems. Current antiulcer treatments focus on inhibition of acid secretion, acid neutralization and or target Helicobacter pylori (H. pylori) which are accomplished by H2 antihistamines or anticholinergics or proton pump inhibitors [2]. It is believed that an increased intake of herbal tailored medicine or food rich in natural antioxidants lowers risks of gastric abrasion and concomitantly peptic ulcer.

However, they can cause adverse effects such as blurred vision, dry mouth, constipation,gynecomastia and galactorrhoea while Proton pump inhibitors have been reported with serious renal problems. The *I. Reniformis chois* are traditionally employed as medicinal herb in Africa, India, and other nations. It is used to treat variety of illnesses and conditions in Ayurveda and traditional

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Chinese medicine. It is found as creeper perennial all through the India, extraordinarily in moist areas across in Gujarat, Bihar, Maharashtra, West Bengal, Goa, Karnataka and Tropical Africa. Phytochemical investigation of the plant have showed the presence of resin, glycosides, reducing sugars, starch, caffeic, pcoumaric, ferulic and sinapic acid esters. In the indigenous system of medicine, Ipomoea reniformis has been used for treatment of inflammation, epilepsy, diuretic, laxative etc[3]. A little pharmacological investigation has been carried out on this plant. But a lot more can still be explored and utilized. Based on the fact that gastric ulcer induction involves oxidative stress and inflammation or microbial infection the current study examines the acid neutralization potential and antioxidant effect of this plant extract[4]. The literature study showed that *I. reniformis* has numerous activities but antiulcer activity study needs to document for the researcher society, therefore we have selected this plant for present study.

2. Materials & Method:

Collection of plant materials and Authentication:

The whole plant of Ipomoea reniformis collected from the Gondia district of Maharashtra during November and was authenticated by botanist Dr. Praveen Kumar Joshi (HOD and Professor) of Shri N. P. A. Government Ayurvedic College, Raipur (C.G.) and specimen were submitted for further study. The leaves were subjected to shade dry for pulverized using mixer grinder into coarse powder and stored in air tight container for chemical evaluation and simultaneous extraction.

***Determination of physical constants*:**

The dried leaf powder were subjected to determine the Loss on drying at 110ºC, Ethanol Soluble Extractive, Chloroform Soluble Extractive, Ether Soluble Extractive, Carbinol Soluble Extractive, Water Soluble Extractive, Total Ash, Water Soluble Ash, Acid Insoluble Ash following protocols.

***Phytochemical screening*:**

The coarse powder was extracted with selected solvents here it was ethyl acetate (EA), chloroform (CE), and ethanol (EE) in a Soxhlet extractor. Hydroalcoholic (HE) (50%v/v) extract was prepared by maceration process[5]. The solvent was completely removed by distillation and dried in a vacuum desiccator. The standard extracts obtained were then stored in a refrigerator at 4°C for further use.

Detection of Carbohydrates: All four extract were tested for Molisch's Test, Fehling's Test, Benedict's test and Test for Starch was conducted following standard procedure laid down in standard references literature.

Test for Proteins and Amino Acids: Small quantities of different extracts were dissolved in few ml of distilled water and subjected to Ninhydrin, Biuret, Millon, Xanthoproteic test, test with tannic acid and heavy metals.

***Test for Alkaloids*:** Small amount of various extracts were separately stirred with a few ml of dilute hydrochloric acid and filtered. The filtrates were tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent and Tannic acid.

***Test for Glycosides*:** A small amount of the all different extracts were dissolved separately in 5ml of distilled water and filtered[6]. Another portion of the extracts were hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to Legal's, Baljet's, Borntrager's, Keller-Killiani's tests and for the presence of Cyanogenetic glycoside.

***Test for Phytosterols*:** All four extracts were refluxed with 0.5N alcoholic potassium hydroxide until the saponification was complete. The saponification mixture was diluted with distilled water and extracted with petroleum ether[7]. The ethereal extract was evaporated and unsaponification matter was subjected to Liebermann's, Liebermann – Burchard's and Salkowski's test.

***Test for Flavonoids*:** The different extracts were separately dissolved in ethanol and then subjected to the Ferric Chloride Test, Shinoda's Test, Fluorescence Test, and Reaction with Alkali and Acid.

***Test for Tannins and Phenolic Compounds*:** The extracts were dissolved in distilled water and filtered. The filtrates were treated with various reagents like 5% ferric chloride solution, copper sulphate solution, lead acetate solution, potassium dichromate solution and potassium ferricyanide solution to detect the presence of phenolic compounds.

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Test for Volatile Oil: A small amount of coarsely powdered extracts was taken in a Cocking- Middleton's apparatus and distilled for 6 hours. Separation of volatile oil during this period indicating the presence of volatile oil.

***Determination of Total Phenolic Content*:**

The total phenolic levels in the crude extracts were determined using Folin-Ciocalteu reagent and external calibration with gallic acid. Exactly 0.2 mL of extract solution and 0.2 mL of Folin-Ciocalteu reagent were mixed thoroughly.

After an interval of 4 min, 1 mL of 15% Na2CO3 was added, and mixture was allowed to stand for a time period of 2 h at room temperature. The measurement of absorbance was taken at 760 nm using a UV-visisble spectrophotometer[8]. Total phenolics concentration was calculated as mg of gallic acid equivalent using an equation obtained from gallic acid calibration curve. The determination of total phenolic compounds was carried out in triplicate, and the results were averaged.

Determination of Total Flavonoids

The flavonoids content of *I. reniformis* extracts was determined using spectrophotometric method.

The sample contained 1 ml of ethanolic solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λmax = 415 nm. The samples were prepared in triplicate for each analysis, and the mean value of absorbance was obtained[9]. The same procedure was repeated for the standard solution of rutin, and the calibration line was constructed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract).

Acid neutralization properties

All the extracts were further diluted for evaluation of Acid neutralizing capacity in concentration 100mg/ml prepared in ethanol and demonized water. The artificial gastric acid was prepared by 2g of NaCl and 3.2 mg of pepsin enzymes dissolved in 500 ml distilled water. Hydrochloric acid (7.0 ml) and adequate water were added to make a 1000 ml solution of artificial gastric acid. The pH of the artificial gastric acid solution was adjusted to 1.20. The pH of each diluted extract solution was determined at temperatures ranging from 25ºC to 37ºC. The pH values of the sodium bicarbonate as standard (SB) and water was also determined for comparison[10]. The Acid neutralization capacity was evaluated in vitro by using the titration method involved preparation of 90mL of each test extract solution and placed in 250mL beaker and warmed to 37ºC with aeration. A magnetic stirrer was continuously run at 30 rpm to mimic the stomach movements. The test samples were titrated with artificial gastric juice to the end point of pH 3. The consumed volume (V) of the artificial gastric juice was measured. The total consumed H+ (mmol) was measured as 0.063096 (mmol/ml) × V (ml).

In vitro Antioxidant Activity

Many in vitro models which determine the antioxidant activity have been reported. Therefore, it is not practical to compare one method to another one. In general, *in vitro* antioxidant tests using free radical traps are relatively straight forward to perform[11]. Among free radical scavenging methods, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method and Superoxide radical scavenging activity is cheap and easy to perform.

***DPPH Scavenging Activity*:** The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenylβ-picrylhydrazyl / DPPH) is characterized by the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize. The delocalization of electron results in deep violet color, characterized by an absorption band in ethanol solution at about 517 nm.

When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color. To assess the antioxidant potential by free radical scavenging of the test samples, the change in optical density of DPPH radicals is observed[12]. The plant extracts and fractions in different concentration (0.2 ml) are diluted with methanol, and 2 ml of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

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DPPH radical scavenging Activity =

$$\frac{Abs_{Control} - Abs_{Sample} \times 100}{Abs_{Control}}$$

***Superoxide Radical Scavenging Activity Method*:** Superoxide radicals are generated *in vitro* by non-enzymatic system and determined spectrophotometrically (560nm) by nitro blue tetrazolium (NBT) photo reduction method[1]. The assay mixture consists of 6.6 mM EDTA containing 3 µg of NaCN, 2 µM of riboflavin, 50 µM of NBT, crude extract, and 67 mM of phosphate buffer (pH 7 .8) in a final volume of 3 mL. The optical density at 560 nm was measured before and after 15 min illumination[13]. The superoxide radical scavenging activity of the crude extracts is expressed in inhibitory concentration 50% (IC_{50}) values.

***Isolation and Purification of Active Constituents by Column Chromatography*:**

Chromatographic techniques have significant role in natural products chemistry as well as contribute dramatically in the discovery of novel and innovative compounds of pharmaceutical and biomedical importance[14]. The dried extract was mixed with silica gel procured from Sigma-Aldrich, Toluca, Mexico, previously activated at 120 °C for 1 h in a drying recirculation oven. A glass chromatography column was filled with the silica gel–acetonic extract mixture[15]. Different solvents (hexane, hexane–ethyl acetate, ethyl acetate, ethyl acetate–methanol and methanol) were used as the mobile phase to recover consecutive 100 mL fractions from the packed column. The fractions were concentrated on rotary evaporator, placed in glass vials and analyzed by thin-layer chromatography (TLC). The fractions whose components showed the same level of displacement in the plate were pooled and placed in an air recirculation oven at 40 °C to evaporate solvent residues. The separation of bands occurs as the column develops[16]. When the edge of the first band (yellow) reached the lower part of the column, the various bands were drawned and labelled. The separations with that observed by TLC with the same solvent were compared. Each fraction was concentrated to a small volume by evaporation for analysis by TLC.

Thin Layer Chromatography

Thin Layer Chromatography was performed on silica gel GF 254 precoated (Merck) plates. The TLC plates of an appropriate size (05 cm x 10 cm) was used. The Mobile Phase used was solvent system prepared by mixing Toluene: Ethyl acetate: Formic acid (5:4:1) as it suits the polarity of the extracts. The mobile phase was poured into the developing chamber to a depth of about 0.5 cm and allowed to equilibrate with the solvent vapor by closing it and waiting for a few minutes[17]. The spotted TLC plates were placed vertically in the developing chamber. When the solvent front reached near the top line (about 1 cm from the top edge), the chromatoplates were removed from the chamber and marked immediately then dried and visualized under a UV lamp (254nm). Rf value of each extract and their spots was calculated for Partition Coefficient (Rf values) by formula = distance travelled by the sample (cm)/distance travelled by the solvent (cm)[18]. The Rf values and spot patterns of the four extracts were analyzed for similarities and differences in their chemical composition and the color, intensity, and number of spots for each extract were recorded. Spots with maximum similarities was isolated using methanol and further subjected to spectral analyses such as UV- Visible spectroscopy (UV), Infrared (IR), Mass spectrometry (MS) and Nuclear magnetic resonance (NMR) to elucidate the chemical structure of isolated compounds.

Four spots at Rf value 0.47, 0.6, 0.88, 0.95 were observed. One spot of Rf value 0.47 was scrapped and put in Petri dish and methanol was added and filtered[19]. The filtrate was evaporated to obtain one sample i.e. Isolated compound V (R*f*: 0.47), may be Scopoletin. The sample was then subjected to CoTLC, chemical test and spectroscopic studies for further identification and confirmation.

Structure Elucidation of isolated constituents by UV, IR, NMR and Mass Spectra.

UV- Visible Spectroscopy

The isolated compound from the chromoplates was dissolved in methanol and filtered. The filtrate obtained was evaporated to obtain pure isolated compound[20,21]. The spectrum characteristics were measured after standardizing the procedures and analysis was confirmed by carrying out triplicate analysis at 200-

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800 nm. Standard Scopoletin in methanol was used for reference comparisons.

Nuclear Magnetic Resonance Spectroscopy

The crystals of all the four extracts were examined by proton nuclear magnetic resonance (1H NMR) spectroscopy, using deuterated acetone (acetone-d6; Sigma- Aldrich, Toluca, Mexico) to solubilize the crystals, in a 400 MHz NMR spectrometer (Jeol, Tokyo, Japan). The acquired spectra were analyzed using MestReNova 2009 software (version 6.0.2-5475; Mestrelab Research S.L., Santiago de Compostela, Spain).

Infrared Spectroscopy

The crystals of the isolated compound was ground in a mortar to reduce the particle size and analyzed using a infrared spectrometer (Frontier, Perkin Elmer, Norwalk, CT, USA) at 25 ± 2 °C in DMSO using TMS as an internal standard.

***Mass spectroscopy*:**

Mass spectra of isolated Compound I shows M-1 peak at 191.10 which indicate that their molecular weight near 191 to 192.

3. Results and discussion

There are nearby 24 Ipomoea species in the state of Maharashtra, India and more than 60 in India. An Ipomoea Reniformis medicinal plant was selected for the study based on review of the literature.

Physical Constant: The physical constant of dried leaf coarse powder was evaluated and Moisture content (loss on drying) was 1.15 ±0.02, Total solid content: 98.85 ±0.04, Ash values in the powder: 4.91 ±0.14, Acid-insoluble ash: 0.93 ±0.37, Sulfated ash: 1.31 ±0.35, Water-soluble extractives: 3.28 ±0.27 and Alcohol-soluble extractives: 4.02 ±0.22 determined using standard literature procedure and the results depicted in table:1.

Table: 1 Physico-chemical parameters of *I. reniformis* (Chois) plants leaves powder

Parameters	Values (% w/w)
% Moisture content in powder	1.15 ±0.02
% Total solid content	98.85 ±0.04
Total Ash values in the powder	4.91 ±0.14
Acid-insoluble ash	0.93 ±0.37
Sulfated ash	1.31 ±0.35
Water soluble Ash	3.28 ±0.27
Alcohol soluble Ash	4.02 ±0.22

Values express in mean ± SD. SD: Standard deviation

***Organoleptic properties and% extractive yield*:** The dried coarse plant leaves powder was extracted in soxhlet extractor with Chloroform, Ethyl acetate, Ethanol and Ethanol: Water (50:50) for about 10-12h at 30^0C. Extracts obtained were dried using evaporator at 30-35^0C to obtain a crude residue. The yield of chloroform extract (CE) was 3.32gm, ethylacetate extract (EA) was 3.32gm, ethanolic extract (EE) was 5.58 gm and Hydroalcoholic extract (HE) was 2.47gm and nature and colour of all extract are represented in table 2.

Table: 2 Nature, color, and % extractive yield of *Ipomoea reniformis* extract.

Solvent used	Nature	Color	Yield of Extract	
			gm	%
Chloroform(CE)	Smooth	Greenish	1. 07	3. 56
Ethyl Acetate(EA)	Smooth	Light	0. 58	1. 93
Ethanol (EE)	Sticky	Dark	3. 48	11. 06
Hydroalcoholic (HE)	Amorphous	Light	2. 13	7. 12

***Phytochemical Evaluation*:** The extracts (CE), (EA), (EE) and (HE) screened showed presence of phytochemical such as alkaloids, glycosides, tannins, Flavonoids, Coumarins, Cardiac Glycosides, Saponins, proteins and amino acids etc. represented in table.

Table: 3 Qualitative analysis of Phytochemical of *Ipomoea reniformis* extract

Phytochemicals Analysis	Ethyl Acetate	Ethanol	Chloroform	Hydroalcoholic
Alkaloids	++	++	+	--
Glycosides	+++	+++	+++	+++
Flavonoids	+++	+++	+++	+++
Coumarin glycosides	+++	+++	--	++

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Anthraquinone Glycosides	++	++	--	--
Cardiac Glycosides	++	++	++	++
Tannins	+++	+++	++	++
Steroids	++	++	++	++
Saponins	++	++	--	++
Resins	++	++		++
Terpenoids	++	++	++	++
Carbohydrates	+++	+++	+++	+++
Proteins	+++	+++	--	+++
Amino acids	++	++	--	++

***Total Phenolic Content*:** The Folin-Ciocalteu reagent was used to measure the total phenolic content in the crude extracts, gallic acid was used for external calibration and absorbance was measured at 760 nm. An equation derived from the gallic acid calibration curve, the total phenolic content was determined (CE) 6.21 ±0.13, (EA) 21.40 ±0.32, (EE) 28.12 ±1.08, and (HE) 16.84 ±0.19 in milligrams of gallic acid equivalent. Total phenolic compounds were measured in triplicate, the findings were averaged summarized in table.4.

Table: 4 Phenols content (as mg GA/g equivalent) of extracts of *Ipomoea Reniformis*

SN	Extracts	mg GA/g of extract
1	Chloroform	6.21 ±0.13
2	Ethyl Acetate	21.40 ±0.32
3	Ethanolic	28.12 ±1.08
4	Hydroalcoholic	16.84 ±0.19

***Total Flavonoids content*:** The spectrophotometric approach was used to quantify the amount of flavonoids present in extracts of *Ipomoea reniformis* as mg of RU/g. The Total Flavonoids content as represented in Table 5 was found to be (CE) 52.32 ±0.69, (EA) 59.29 ±1.87, (EE) 59.09 ±1.87, and (HE) 24.54 ±1.87 in mg of RU/g of extract equivalent.

Table: 5 Flavonoids content (as mg of RU/g of extract) of *Ipomoea reniformis*

Sl. No.	Extracts	mg of RU/g of extract
1	Chloroform	52.32±0.69
2	Ethyl Acetate	59.29±1.87
3	Ethanolic	59.09±1.87
4	Hydroalcoholic	24.54±1.87

According to the results of the experimental work it was proven that, *Ipomoea reniformis extracts* has antioxidant action. It could be considered from the results that the antioxidant activity of any extract maybe influenced by the phenolic and flavonoids concentration.

***Acid neutralization capacity*:** All the diluted extracts were estimated for its pH at temperatures ranging from 25ºC to 37ºC. The pH value of CE was 1.54, EA was 1.56, EE was 1.53 and HE 1.42. The pH values of water was 1.39, standard Sodium bicarbonate (SB) was 1.72 determined for comparison. The *in vitro* Acid neutralization capacity was calculated by titrating suitably diluted extract against artificial gastric juice of pH 3 to the end point.

Table:6 Represent pH values, Consumed volume of artificial gastric juice mL and mmole of H+ of Ipomoea extract.

SNo	Extract	pH value	Consumed volume of Artificial gastric juice mL	mmole of H+
1	Water	1.39	1.30 ± 0.02	0.07 ± 0.00
2	Standard (SB)	1.72	32.44 ± 0.59	2 .15 ± 0.03
3	Chloroform Extract (CE)	1.54	7.24 ± 0.08	0. 5 ± 0.02
4	Ethyl Acetate Extract (EA)	1.56	10.56 ± 0.09	1.78 ±0.01

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5	Ethanol Extract (EE)	1.53	9. 36 ± 0.09	1.56 ± 0.01
6	Hydroalcoholic Extract (HE)	1.42	7.38 ± 0.08	0.6 ± 0.02

Data are presented as mean ± SEM (N=6) P* ≤ 0.05 when compared with water.

- The pH values of CE, EA, EE and HE extracts were found to be 1.54, 1.56, 1.53 and 1.42 respectively. The pH values of water and Sodium bicarbonate solutions were 1.39 and 1.72.
- The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, Sodium bicarbonate, CE, EA, EE and HE extracts solutions were found to be 1.3 ±0.02, 32.44 ±0.59, 7.24 ±0.08, 10.56 ±0.09, 9.36 ±0.09 and 7.38 ±0.08 respectively.
- The consumed H+ were 0.07 ±0.00, 2.15 ±0.03, 0.5 ± 0.02, 1.78 ±0.01, 1.56 ±0.01 and 0.6 ±0.02 mmol respectively.

The neutralization capacities of all the extracts were lesser than that of Sodium bicarbonate but significantly better for Ethyl acetate extract and Ethanol extract which indicates significant antacid potency.

In Vitro Antioxidant Activity

In DPPH method all the extracts of *Ipomoea reniformis* have significantly reduced the DPPH radicals in a concentration dependent manner. DPPH assay assessed the free radical scavenging property of all extract by comparing the Inhibitory concentration (IC_{50}) and standard Vitamin C as shown in Table 7 and Figure 1. EE showed potent antioxidant activity with IC_{50} value of 52.24 µg/ml and standard 205.08µg/ml. In superoxide radical scavenging method, the EE again showed highest activity 96.66 µg/mL⁻1 among all the extract used followed by HE with a value of 88.01 µg/mL^{-1}.

Table:7 Effect of various extracts of *I. reniformis* on IC50 values by DPPH and Superoxide radical scavenging activity.

SN	Extract / Standard	IC_{50} µg/mL^{-1}	
		DPPH method	**Superoxide Radical Scavenging Method**
1	Chloroform extract	59. 05µg/ml	54.01 ±0.59
2	Ethyl Acetate extract	91.92 µg/ml	47.12 ±1.23
3	Ethanol Extract	52.24 µg/ml*	96.66 ±1.41
4	Hydroalcoholic	57.89 µg/ml	87.42 ±0.89
5	Vitamin C (Standard)	205. 08 µg/ml	165 ±1.02

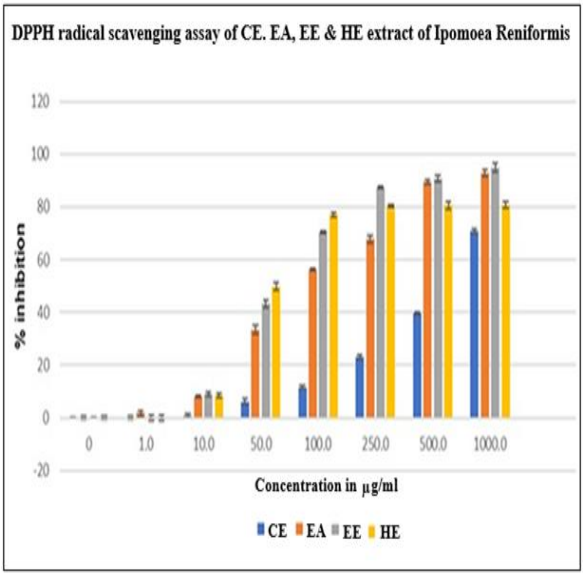

Figure 1: The standard Vitamin C exhibited better results with lower IC_{50} values in both DPPH method and Superoxide Radical Scavenging method. The phenolic and flavonoids content of ethanolic extract was much higher than other extracts used for the study, and this might be the main reason behind its antioxidant activity.

***Isolation and Purification of Active Constituents*:**

The EA extract which has shown higher antioxidant and acid neutralizing ability was fractionation by TLC. Four spots at Rf value 0.48, 0.65, 0.84, 0.86 were found identical. One spot of R*f* value 0.84 was scrapped and dissolved in methanol and evaporated. The separated as compound I using is subjected to identification by using UV, NMR, IR and Mass spectrometry.

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Characterization and confirmation of isolated compound:

UV-Visible spectroscopic analysis of isolated Compound I: UV spectrum of the Methanolic solution has characteristic bands at 339.55nm and 295.22nm. On addition of 2-4 drops of 2M NaOH, bathochromic shift was observed.

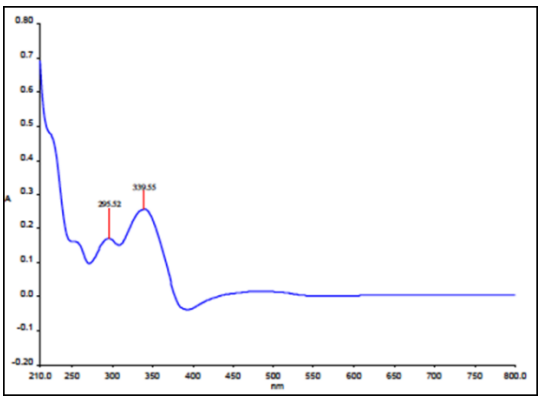

Figure 2: Showing UV spectrum of the drug

FTIR Analysis of Isolated Compound: In the IR spectral analysis, the peak at 3341.44 cm-1, a broad band may be due to the result of O-H stretching vibrations of phenols OH group. The peak at 2875.05 cm-1 showed C-H Stretching. The peak at 1703.42 indicates the presence of -C=O a Carbonyl group. The peak at & 1606.75 showed the presence of -CH=CH group. The peak at 1568.83 and 1511.16 indicates the presence of benzene ring. The peak at 861.50 showed the presence of disubstitution of benzene ring in isolated compound I.

Table:8 Shows the data obtained from FTIR Spectroscopy and possible functional groups present. Scopoletin IR study gave following results:

Peaks (cm-1) of isolated Compound-I	**Functional group**
3341.44	**-OH** group present
2875.05	**C-H** group present
1703.42	**C=O** group present
1606.75	**CH=CH** group present
1568.83	**Benzene** ring present
1511.16	**Benzene** ring present
861.50	Due to distribution of benzene

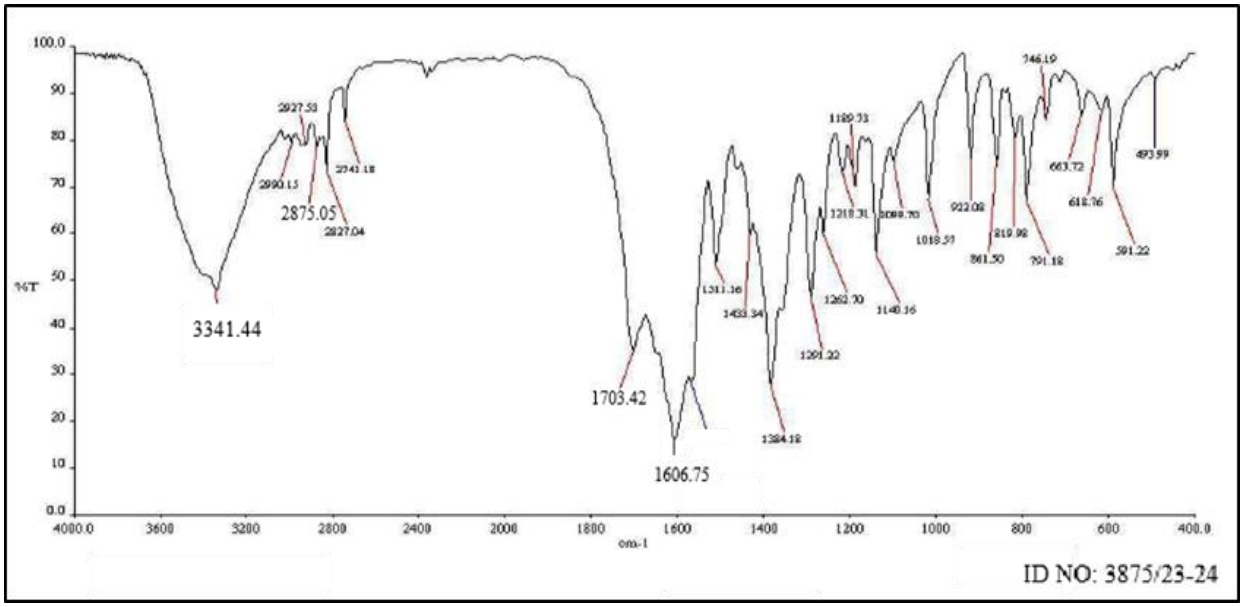

Figure 2: Showing IR spectrum of Compound-I

NMR spectroscopy

The 1H NMR spectrum of isolated Compound I showed two doublets with coupling constant of 9.2 Hz at δ 6.22 and 7.88 ppm, which were assigned as H-3 and H-4, isolated compound I.

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Table 9 : Shows the data obtained from NMR Spectroscopy

No. of H atom	Standard Scopoletin		Isolated Compound I from the fraction	
	δ value, ppm	Integration, Multiplicity (J, HZ)	δ value, ppm	Integration, Multiplicity (J, HZ)
3	6.23	1H, d(9.2)	6.22	1H, d(9.2)
4	7.88	1H, d(9.2)	7.88	1H, d(9.2)
5	7.14	1H, S	7.13	1H, S
8	6.79	1H, S	6.79	1H, S
C-6-OMe	3.93	3H, S	3.93	3H, S

***Mass spectroscopy*:**

Mass spectra of isolated Compound I shows M-1 peak at 191.10 which indicate the molecular weight (Figure 3).

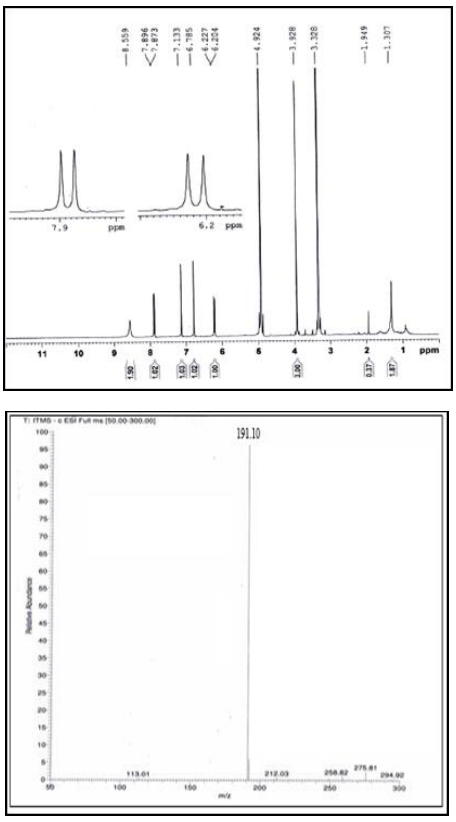

Figure 3 Showing Mass spectrum of Compound-I.

4 Conclusion:

Herbal drugs show promising results in treating a variety of conditions, ranging from common colds to chronic diseases such as diabetes and hypertension. Eco-friendly approach should be aligned with modern research methods in growing interest of sustainable and ethical healthcare practices. The present phytochemical and pharmacological investigation of Ipomoea reniformis Chois from the Convolvulaceae family highlights its significant acid neutralizing capacity and free radical scavenging potential thus can be employed in the treatment of ulcers. However, further studies, including clinical trials and mechanistic studies, are required to fully elucidate the underlying mechanisms and to validate its efficacy and safety in humans. The findings of this study underscore the therapeutic potential of Ipomoea reniformis as an antiulcer agent. Its rich phytochemical profile, coupled with its pharmacological properties, opens up new avenues for its application in the management of peptic ulcers, particularly as a natural alternative to synthetic drugs with fewer side effects.

Conflict of Interest Statement: The authors declare no conflict of interest.

Ethical Statement: Not Applicable

Funding: None

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