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Research paper

Antioxidant activity guided isolation of a coumarin compound from *Ipomoea pes-caprea* (Convolvulaceae) leaves acetone extract and its biological and molecular docking studies

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ABSTRACT

Introduction: Several modern drugs have been derived from plants, which contain numerous biologically active metabolites. *Ipomoea pes-caprea* (F: Convolvulaceae) is used in traditional medicine to treat several ailments. The present study aimed to investigate the pharmacological properties and the biological applications of *Ipomoea pes-caprea*.

Methods: The phytochemical screening analysis, estimation of phytoconstituents, antioxidant, and antibacterial activities were conducted. Based on the results of preliminary studies the bioactive extract was subjected to GC–MS analysis, and activity guided isolation of bioactive compound obtained. The isolated active principle was subjected to various spectral analyses such as: UV, FT-IR, LC–MS and ^{1}H, ^{13}C, DEPT, HMBC, and HSQC NMR to elucidate the structure and analyze its biological potential.

Results: The results of phytochemical screening indicated the presence of various phytochemicals in *Ipomoea pes-caprea* extracts. The acetone extract possesses a significantly high amount of metabolites with antibacterial as well as antioxidant capacity, with low IC_{50} values. Results of acetone extract GC–MS analysis revealed that the presence of several bioactive compounds where 2-phenyl-4*H*-chromen-4-one was predominant. Antioxidant activity guided isolation of acetone extract resulted in the isolation of a coumarin compound (5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one) and the structure was confirmed using various spectral studies. The isolated 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one exhibited significant antioxidant, antimicrobial and antiproliferative activities. In addition, the molecular docking study revealed that the isolated coumarin is a good inhibitor of the angiopoietin-2.

Conclusions: The present study concludes that the isolated compound (5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one) may harbor significant health benefits and it may serve as a good molecular template in pharmacology for the development of new drugs.

1. Introduction

Herbal plants are excellent sources of natural antioxidants, which may act as potential drugs for various diseases such as cancer, autoimmune disorders, inflammatory conditions, and diabetes [1,2]. Plants contain several valuable phytochemicals including phenolics, flavonoids, coumarin, carotenoids, and tocopherols which are responsible for their antioxidant potential. Antioxidants reduce the oxidative damage caused by reactive oxygen species (ROS) [3]. The use of plants may have a beneficial role in the protection of oxidative stress, which plays a major role in chronic and degenerative ailments. Nowadays, searching for the potent antioxidant compounds from various sources is of increasing interest in the pharmaceutical area because they can inhibit the free radical reactions, thereby preventing the development of oxidative stress-related diseases [2].

Pathogenic microbes are the major threats to public health which causes morbidity and mortality [4]. Nowadays, several life-threatening microbes have acquired resistance to many antibiotics that are currently available in the markets [5]. Besides the resistance problems, many synthetic antibiotics are of high cost and reported to have various side effects such as hypersensitivity, reduction in the beneficial gut microbes and immune suppression [6]. Hence, there is an urgent need

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to identify new, safer, high potential, and wide-spectrum antimicrobial molecules from plants [7].

According to the World Health Organization, around 80% of people in the globe rely on plant-based medicines for their primary health care needs [8]. The compounds discovered/derived from the plant kingdom are known to have many physiological properties in modern medicine [9]. Thus, there has been growing research interest in the isolation of potent antioxidant compounds from plant sources and synthesis of its derivatives [10]. Molecular docking is a computational method, which has a special place in the designing of new highly potent drugs [11]. Particularly, in the study of interactions between small molecules (ligand) and target proteins that use to predict the activation or inhibition of the enzymes. The docking technique provides knowledge about tentative binding parameters of the ligand-receptor complex in ahead of time [12].

Ipomoea pes-caprae (F: Convolvulaceae) is a medicinal plant which is used in traditional medicine to treat various diseases, especially, oxidative stress-related diseases [13,14]. It is traditionally used in the treatment of inflammation, colic, dysentery, internal pain, skin diseases, gastrointestinal tract related problems, diabetes, and as the antidote for jellyfish poison, *etc*. [15]. It is widely distributed throughout the tropical and subtropical regions and coastal beaches of North and South America, India, Asia and Australia [16]. *Ipomoea pes-caprae* is commonly known by a variety of names, dopatilata, railroad vine, goat's-foot, beach morning glory, and salsa-da Praia [17,18]. *Ipomoea pes-caprae* has various biological activities such as anticancer, antioxidant, antidiabetic, antispasmodic, anti-inflammatory, *etc*. [19]. A few biologically active compounds, namely, eugenol, 4-vinylguaiacol, actinidols, (*E*)-phytol, betulinic acid, isoquercitrin, stoloniferin, and pescaprosides A and B have been isolated from *Ipomoea pes-caprae* [20,21]. Hence, the present investigation aimed to study the phytochemical, antioxidant, antibacterial profile of *Ipomoea pes-caprea* leaves extracts and isolate its antioxidant principles with special reference to DPPH radical scavenging activity. The antioxidant, antibacterial, antiproliferative potential of the isolated compound was evaluated and was docked with angiopoietin-2.

2. Materials and methods

2.1. Chemicals

All the chemicals and solvents used in this study were purchased from Sigma Aldrich and HiMedia companies and which were > 95% pure and/or analytical grade. Distilled water used in this investigation was procured from Mercury scientific chemicals, Salem.

2.2. Plant collection

Healthy leaves of *Ipomoea pes-caprae* were collected from the Sarabanga river bank (289 m MSL, latitude 11°44′ 8.145″N, longitude 78°02′14.568″E), Omalur, Salem District, Tamil Nadu, India. The plant material was collected during the flowering season (July to December, 18–30 °C). The collected plant sample was identified and authenticated by the Botanical Survey of India (Reference number: BSI/SRC/5/23/2014-15/Tech.1458), Coimbatore, Tamil Nadu, India. The voucher specimen of the collected plant was deposited the Phytochemistry Laboratory, Department of Chemistry, Periyar University, Salem, Tamil Nadu, India. Collected leaf material was examined for insect damages and fungal infections; washed with tap water prior to sterile distilled water, shade dried at room temperature for three weeks and powdered using an electric grinder.

2.3. Extraction of plant materials

About 5 kg of powdered plant material was sequentially extracted with various organic solvents (*n*-hexane, chloroform, acetone, ethyl acetate, methanol, and water) in an increasing polarity manner using a Soxhlet apparatus until the effluent solvent become colorless. All the extracts were filtered through a Whatman (No.1) filter paper and concentrated under reduced pressure at 40 °C using a rotary vacuum evaporator which yielded 2.53%, 2.81%, 6.03%, 3.89%, and 8.27% of *n*-hexane, chloroform, acetone, ethyl acetate, and methanol extracts, respectively. The concentrated crude extracts were maintained at 4 °C for further studies.

2.4. Phytochemical analysis

2.4.1. Qualitative phytochemical analysis

Preliminary phytochemical screening analysis of various crude extracts of *Ipomoea pes-caprae* leaves was done by standard procedures described by Evans et al. [22].

2.4.2. Quantitative analysis of phytoconstituents

2.4.2.1. Estimation of total phenol content. The phenolic content of plant extracts was estimated by the Folin-Ciocalteu method, as described by Barreira et al. [23].

2.4.2.2. Estimation of total flavonoid content. The flavonoid content of the plant extracts was measured by a colorimetric method [24].

2.4.2.3. Estimation of total carbohydrate content. The total carbohydrate content of the extracts was determined as per Anthorne method described by Hedge et al. [25].

2.4.2.4. Estimation of total protein content. Lowry's method was used to determine the protein content of the extracts [26].

2.4.3. In vitro antioxidant activity

Various concentrations (200, 400, 600, 800 and 1000 µg/mL) of extracts were examined for different types of radical scavenging ability. Ascorbic acid and butylated hydroxyanisole (BHA) used as standard antioxidants for all analysis.

2.4.3.1. DPPH radical scavenging activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging potential of plant extracts were examined according to the method of Chew et al. [27]. The percentage of DPPH radical scavenging activity (DPPH RSA) was calculated using the following formula,

$$DPPH\ RSA(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4.3.2. Hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity of plant extracts was determined by the Fenton method [28]. The hydroxyl radical scavenging activity was calculated by the following equation,

$$Hydroxyl\ RSA(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4.3.3. Nitric oxide radical scavenging activity. The nitric oxide radical scavenging ability of *Ipomoea pes-caprae* extracts was estimated as per the method of Sfahlan et al. [29]. The percentage of nitric oxide radical scavenging capacity was calculated by the following equation,

$$Nitric\ oxide\ RSA(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4.3.4. Superoxide radical scavenging activity. The superoxide anion radical scavenging potential of the extracts was examined as per the

Table 1
DPPH antioxidant activity IC_{50}/EC_{50} value of the fractions.

S. no	DPPH assay*							
	First fraction name	IC_{50} values (μg/mL)	Second fraction name	IC_{50} values (μg/mL)	Third fraction name	IC_{50} values (μg/mL)	Fourth fraction name	IC_{50} values (μg/mL)
1	L-HF1	35.80 ± 0.12^{e}	L-HF2	43.34 ± 0.12^{f}	10 HA	10.33 ± 0.12^{c,d}	1th	10.01 ± 0.25 j
2	L-CF1	24.91 ± 0.13^{b,c}	L-CF2	19.88 ± 0.08^{c}	90 HA	12.35 ± 0.05^{k,l}	2th	09.45 ± 0.08^{g,h}
3	L-EF1	19.51 ± 0.06^{a}	L-EF2	16.25 ± 0.06^{b}	91 HA	12.35 ± 0.05^{k,l}	3th	08.90 ± 0.38^{e}
4	L-AF1	25.43 ± 0.03^{b}	L-AF2	13.84 ± 0.05^{a}	81 HA	09.95 ± 0.12^{a,b}	4th	08.55 ± 0.20^{c}
5	L-MF1	34.10 ± 0.06^{d}	L-MF2	21.84 ± 0.20^{d}	82 HA	11.00 ± 0.09^{f}	5th	08.75 ± 0.16^{d}
6	L-WF1	35.67 ± 0.13^{e,f}	L-WF2	23.76 ± 0.11^{e}	72 HA	10.71 ± 0.16^{d,e}	6th	08.48 ± 0.21^{b}
7					73 HA	10.13 ± 0.01^{c,d}	7th	08.00 ± 0.04^{a}
8					63 HA	10.01 ± 0.02^{c}	8th	09.42 ± 0.14^{g,h}
9					64 HA	11.91 ± 0.22^{j}	9th	09.10 ± 0.05^{f}
10					54 HA	11.25 ± 0.23^{f,g,h}	10th	09.88 ± 0.47^{i}
11					55 HA	11.50 ± 0.08^{i}		
12					45 HA	11.12 ± 0.11^{f,g}		
13					46 HA	09.25 ± 0.24^{a}		
14					36 HA	13.01 ± 0.14^{o,p}		
15					37 HA	12.00 ± 0.11^{k}		
16					27 HA	12.60 ± 0.07^{m,n}		
17					28 HA	12.95 ± 0.00^{m,n}		
18					18 HA	12.85 ± 0.12^{m}		
19					19 HA	13.00 ± 0.31°		
20					09 HA	13.55 ± 0.32^{r}		
21					01 HA	13.24 ± 0.42^{q}		

L-HF1-*n*-hexane fraction-1, L-CF1-chloroform fraction-1, L-EF1-ethyl acetate fraction-1, L-AF1-acetone fraction-1, L-MF1-methanol fraction-1, L-WF1-water fraction-1, L-HF2-*n*-hexane fraction-2, L-CF2-chloroform fraction-2, L-EF2-ethyl acetate fraction-2, L-AF2-acetone fraction-2, L-MF2-methanol fraction-2, and L-WF2-water fraction-2, Third fraction name (100 Hexane: 00 Acetone to 00 Hexane:100 Acetone) and fourth fraction name (1 to 10th).

* The values are mean of triplicates with (±) standard deviation (mean ± S.D. n = 3). Different superscript letters (a–r) in column indicate significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

method of Liu et al. [30]. The percentage of superoxide anion radical scavenging ability was calculated from the following formula,

$$Superoxide\ RSA(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.4.3.5. Reducing power ability. The ferric reducing ability of various extract of *Ipomoea pes-caprae* was analyzed by the method of Yen and Chen [31].

Aforementioned all *in vitro* antioxidant analyses were also carried out for the different concentrations (20, 40, 60, 80 and 100 μg/mL) of the isolated compound.

2.4.4. Antibacterial activity

Two gram-positive bacterial strains, *Streptococcus pneumoniae*, and *Staphylococcus epidermidis*, and four gram-negative bacterial strains *viz*., *Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Salmonella typhi* were used for the antibacterial study. All the selected bacterial strains were clinical isolates which obtained from clinical laboratories (as complimentary), in and around Salem District, Tamil Nadu. *S. typhi*, *K. pneumoniae*, *S. pneumoniae*, and *S. epidermidis* were isolated from blood samples of infected patients. While *E. coli* and *S. flexneri* were isolated from urine and stool samples respectively. The antibacterial potential of the various solvent leaves extracts of *Ipomoea pes-caprae*, and isolated compound also evaluated using the agar well diffusion method, as per the protocol of Srinivasan et al. [7]. A 50 μL of plant extracts (100 mg/mL) and isolated compound (1 mg/mL) were examined for the antibacterial capacity and equal volume of DMSO served as a negative control. 10 μL of ciprofloxacin (1 mg/mL) a standard antibiotic was used as a positive control.

2.5. GC–MS analysis

The result of preliminary antioxidant and antibacterial studies of *Ipomoea pes-caprae* extracts revealed that the acetone extract possesses significant antioxidant and antibacterial potential. Thus, the acetone extract was selected for further analysis like GC–MS, and antioxidant activity guided isolation of bioactive compound(s). The acetone extract was analyzed using a GC–MS (Thermo GC- Trace Ultra Ver: 5.0) equipped with a DB 5- MS capillary standard non - polar column (30 m length × outside diameter 0.25 mm × internal diameter 0.25 μm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) and GC–MS detection, an electron ionization system with ionization energy of −70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min and 1 μl samplewas injected. The temperature was programmed to 70–200 °C at the rate of 10 °C/min and held at 250 °C at 55 min. The ion source temperature was 200 °C. The mass spectrum of the unknown components was compared to Wiley and NIST mass spectrum library. The retention time (RT), name, and structure of the detected compounds present in the acetone extract were ascertained [32].

2.5.1. Antioxidant activity guided isolation of bioactive compounds

Ipomoea pes-caprae leaves acetone extract was found as bioactive extract and subjected to the antioxidant activity guided isolation of bioactive compound(s). All the fractions and subfractions obtained in the isolation process were evaluated for DPPH radical scavenging potential throughout the isolation process, in which fractions show high antioxidant activity with low IC_{50} value again subjected to the further purification process [10]. The acetone extract (50 g) was fractionated using various organic solvents by maceration processes and fractions were named as the respective solvents used for the extraction, such as leaf *n*-hexane fraction-1 (L-HF1), chloroform fraction-1 (L-CF1), ethyl acetate fraction-1 (L-EF1), acetone fraction-1 (L-AF1), methanol fraction-1 (L-MF1) and water fraction-1 (L-WF1). The results revealed that the L-EF1 fraction has a higher antiradical capacity with low IC_{50} value. Thus, the ethyl acetate (L-EF1, 7.8 g) fraction was again fractioned using same organic solvents by maceration processes which yields 6 subfractions namely, *n*-hexane fraction-2 (L-HF2), chloroform fraction-

2 (L-CF2), ethyl acetate fraction-2 (L-EF2), acetone fraction-2 (L-AF2), methanol fraction-2 (L-MF2) and water fraction-2 (L-WF2). The sub-fraction L-AF2 show high antiradical capacity with low IC_{50} value (Table 1).

The L-AF2 (2.1 g) fraction was applied to a silica gel (60–120 mesh) column (60 × 4 cm) to isolate the bioactive principles and subjected to GC–MS analysis. The column was eluted with a solvent gradient mixture of hexane: acetone, in the order of increasing polarity (100:0–0:100) by 5% and the fractions were collected in a glass container and stored at room temperature. The column chromatography yields 21 fractions. A significant antiradical activity with low IC_{50} value was observed in 40:60-hexane: acetone ratio fraction (46-HA) (Table 1). The 46-HA fraction was monitored by TLC to determine the optimal solvent system for further purification and subjected to GC–MS analysis. 46-HA fraction was again subjected to a small size column (15 × 1 cm) chromatography and eluted with 100% hexane followed by a gradient mixture (95:5–100) of hexane: methanol. Totally, 10 fractions were collected among them, a 7th fraction (Hexane: methanol (98.5:1.05 v/v); light yellow powder, 509 mg) showed remarkable DPPH antiradical potential with low IC_{50} value and a single spot in TLC analysis with 0.75 *Rf* value (under UV light and iodine vapor). The TLC developed in *n*-hexane:chloroform:methanol (6:3:1) solvent system as the mobile phase. Thus, the 7th fraction was selected for further spectral analysis. The entire isolation process was elucidated as a schematic diagram in Fig. 1.

Supplementary Fig. 1 shows the HPLC profile of the chemical constituents present in the various fractions of *Ipomoea pes-caprae* leaves. The HPLC chromatogram of the purified compound exposed the presence of a peak, with a retention time of 2.37 min, eluted isocratically with the mobile phase of 50% methanol: 50% water (v/v).

2.5.2. Structural elucidation of active compound

The structural elucidation of the isolated compound was carried out by the data of various spectral analyses such as LC–MS, FT-IR, and NMR. The purity of the isolated compound was studied by an HPLC system. The HPLC analysis was performed using a C-18 column (250 × 4.6 mm, 5μ) in a Shimadzu SPD-20A chromatography apparatus (Reservoir tray, Shimadzu Corporation, Kyoto, Japan). LC–MS analysis was carried out by using a UHPLC-MS system (410 Prostar Binary LC with 500 MS IT PDA Detectors, Varian Inc, USA). The pure compound (~3 mg) was mixed with 100 mg of anhydrous KBr powder to prepare thin disc using pellet making the machine and this pelletized sample FT-IR spectrum was recorded (400 to 4000 cm^{-1} wavelength) using Perkin Elmer, USA, Spectrum RX-I, FT-IR spectrometer. Nuclear Magnetic Resonance (NMR) spectral analyses like ^{1}H-NMR, ^{13}C-NMR, DEPT, HMBC, and HSQC were recorded using a Brucker Avance III 500 MHz NMR spectrometer (Mercury Plus 300 MHz NMR Spectrometer, Varian, USA). The DMSO-d_6 and TMS were used as NMR solvents for isolated compound and an internal standard respectively.

2.5.3. Antiproliferative activity

The antiproliferative activity of the isolated compound was determined by methylthiazolyl diphenyl-tetrazolium bromide (MTT) assay as described by Mosmann, [33] on HCT-116 human colon cancer cells. HCT-116 cancer cells were treated with various concentrations of the isolated compound (1–50 μM/mL). The effect of the isolated compound on the proliferation of HCT-116 human colon cancer cells was expressed as a percentage of cell viability. The IC_{50} value was calculated graphically from the curve plotted for the percentage of cell viability against the concentration of the compound.

2.5.4. Molecular docking study

Molecular docking was performed to study the inhibitory potential of isolated coumarin compound (5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one) against angiopoietin-2 receptor (PDB: 1Z3S) protein by using the AutoDock tool (AutoDock 4.2 by Lamarckian genetic Algorithm) and Cygwin [34,35].

2.5.5. Statistical analyses

Statistical analyses were done in SPPS software (16.0 version). Analysis of variance (ANOVA) was carried out in a completely randomized design and Tukey's multiple range tests were used to compare the significant differences between samples. Values were expressed as a mean ± standard deviation. All determinations were done at least in triplicate, and all were averaged. The confidence limits used in this study were based on 95% ($p < 0.05$).

3. Results

3.1. Phytochemical analysis

3.1.1. Qualitative analysis

The results of the qualitative phytochemical screening analysis revealed the presence of various phytochemicals such as alkaloids, flavonoids, glycosides, terpenoids, phenolics, saponins, carbohydrates, terpenoids, and leucoanthocyanidins in different solvent extracts of *Ipomoea pes-caprae* (Table 2). Acetone extract harbored most of the tested phytochemicals followed by methanol extract. Hence, acetone may be a good solvent to extract the different kinds of phytochemicals from *Ipomoea pes-caprae* leaves.

3.1.2. Quantitative analysis

The outcome of the quantitative phytochemical analysis revealed the diversified quantities of tested metabolites in different solvent extracts ranged from 12.68 to 150.10 μg/mg (Table 3). Moreover, significant-highest amount of all tested metabolites such as, polyphenols (79.35 ± 0.18 μg/mg GAE), flavonoids (150.10 ± 0.63 μg/mg CE), proteins (125.33 ± 1.15 μg/mg BSAE) and carbohydrates (93.35 ± 0.24 GE) were noticed in acetone extract followed by ethyl acetate extract.

3.2. In vitro antioxidant activity

All the tested extracts of *Ipomoea pes-caprae* expressed sustainable to strong antioxidant potential in a dose-dependent manner with considerable IC_{50} values (Table 4 and Fig. 2). However, acetone extract possesses an excellent antiradical potential that significantly inhibited all types of tested radicals. The acetone extract showed maximum radical scavenging potential on superoxide radicals (89%) with lowest IC_{50} value (19.50 ± 0.31 μg/mL) which lower than the standard compounds (ascorbic acid and BHA) followed by hydroxyl radicals (IC_{50} value 20.99 ± 0.03 μg/mL). Methanol and ethyl acetate extracts expressed considerable antiradical activity in all tested methods with sustainable IC_{50} values. Water, hexane and chloroform extracts harbor minimal antioxidant potential with higher IC_{50} values.

3.3. Antibacterial activity

Different extracts of *Ipomoea pes-caprae* exhibited a broad spectrum of antibacterial activity (8–22 mm) against most of the tested pathogens except for the water extract (Table 5). The results of antibacterial activity revealed that the acetone extract has significant antibacterial potential against most of the tested pathogens and the maximum growth inhibition was observed against *Escherichia coli* (22 mm) followed by *Streptococcus pneumoniae* and *Salmonella typhi* (12 mm). Hexane, chloroform and methanol extracts show minimal antibacterial activity against most of the tested pathogens. Water extract showed nil antibacterial capacity against all tested pathogens as depicted Supplementary Fig. 2.

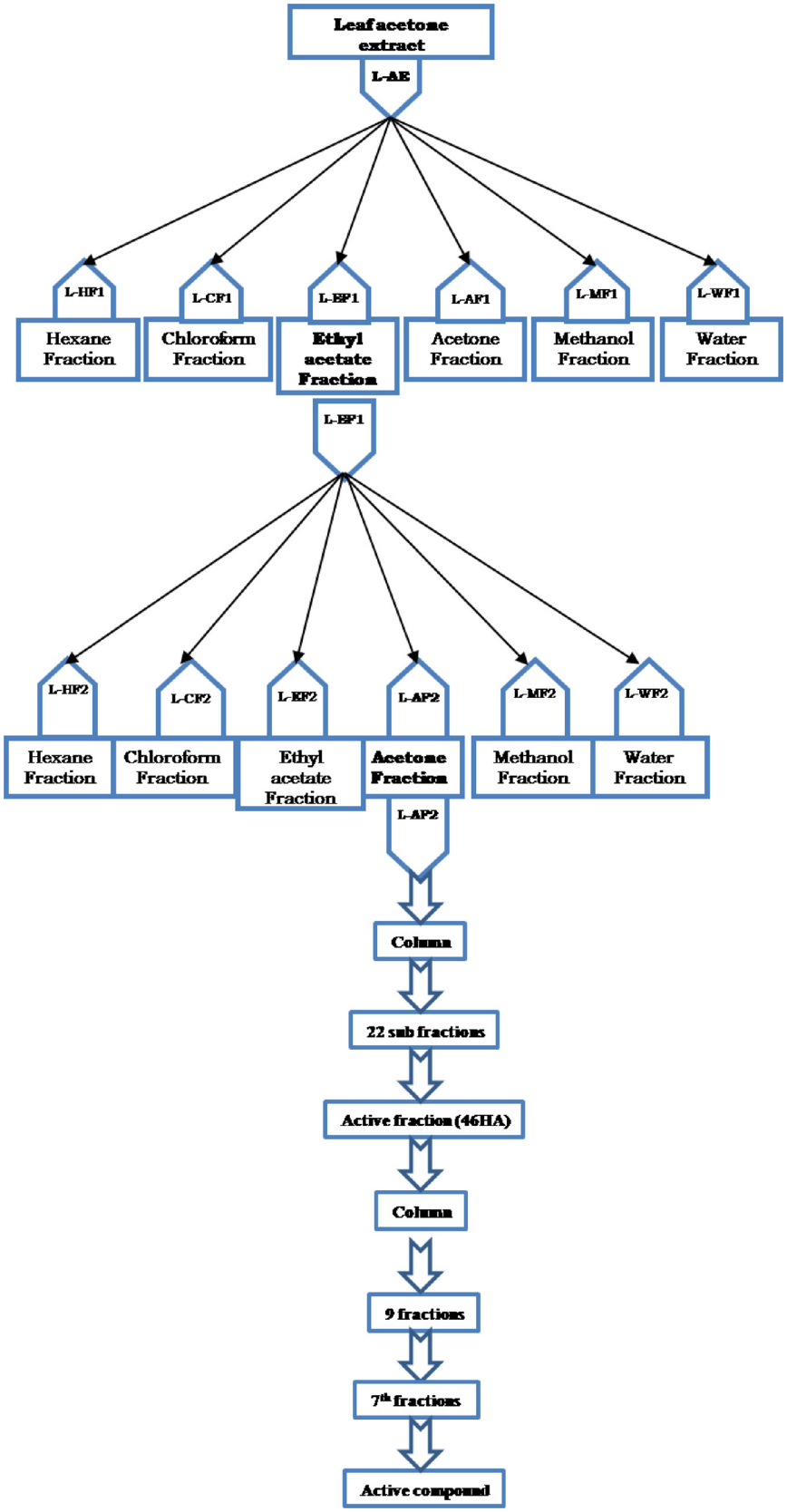

Fig. 1. Schematic diagram of isolation of antioxidant compound.

Table 2
Phytochemical screening of various solvent leaves extracts of *Ipomoea pes-caprae.*

S. no.	Phytochemicals	Extracts					
		Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water
1.	Alkaloids	+	+ +	+	-	-	-
2.	Flavonoids	+ +	+ +	+ +	+ + +	+ +	+ +
3.	Flavonols	-	-	-	+	+	+ +
4.	Glycosides	-	-	+ +	-	+ +	-
5.	Cardiac glycosides	-	-	+ +	-	+	-
6.	Tannins	-	-	-	+ + +	+ +	+ + +
7.	Phenolics	-	-	+	+ + +	+ +	-
8.	Amino acids	-	-	-	+	+	-
9.	Steroids	-	-	+	+ +	+ + +	+
10.	Proteins	-	-	-	+	+	-
11.	Carbohydrates	-	-	-	+ +	+	+ +
12.	Saponins	+	+	-	-	-	-
13.	Terpenoids	-	-	+ + +	+ + +	+ + +	+
14.	Fats/oils	+ +	+ +	+	-	-	-
15.	Leucoanthocyanidins	+	+ +	+ +	+ +	+ +	+ +

+ + + = copiously present, + + = moderately present, + = slightly present, − = absent.

Table 3
Estimation secondary and primary metabolites of various solvent leaves extracts of *Ipomoea pes-caprae*.

Phytoconstituents (μg/mg of extract)	Extracts*					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water
Total phenol content	12.68 ± 0.90^{a}	37.47 ± 0.13^{d}	47.88 ± 0.09^{e}	79.35 ± 0.18^{f}	26.66 ± 0.49^{b}	30.36 ± 0.18^{c}
Total flavonoid content	35.97 ± 0.14^{a}	64.30 ± 0.14^{b}	110.49 ± 0.36^{e}	150.10 ± 0.63^{f}	101.68 ± 0.14^{d}	88.03 ± 0.77^{c}
Total protein content	20.00 ± 0.00^{a}	24.67 ± 1.15^{b}	83.33 ± 1.15^{e}	125.33 ± 1.15^{f}	54.67 ± 1.15^{d}	40.67 ± 1.15^{c}
Total carbohydrate content	31.90 ± 0.08^{c}	21.80 ± 0.37^{a}	47.01 ± 0.42^{d}	93.35 ± 0.24^{f}	76.50 ± 0.08^{e}	27.25 ± 0.49^{b}

* The values are mean of triplicates with (±) standard deviation (mean ± S.D. n = 3). Different superscript letters (a–f) in rows indicates the effectiveness of extracts (f > e > d > c > b > a) with significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

Table 4
Antioxidant activity IC_{50}/EC_{50} values of different leaves extracts and isolated compound of *Ipomoea pes-caprae*.

Assays	IC_{50}/EC_{50} values (μg/mL)*								
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water	Isolated compound	AA	BHA
DPPH RAS	65.86 ± 0.09^{h}	64.12 ± 0.29^{g}	31.38 ± 0.03^{d}	23.78 ± 0.02^{c}	33.50 ± 0.00e	46.40 ± 0.28^{f}	08.15 ± 0.15^{a}	21.78 ± 0.01^{b}	14.48 ± 0.14^{a}
Hydroxyl RAS	50.40 ± 0.24^{g}	58.07 ± 0.06^{h}	38.14 ± 0.00^{e}	20.99 ± 0.03^{b,a}	34.24 ± 0.14^{d}	43.08 ± 0.44^{f}	14.11 ± 0.05^{a}	20.35 ± 0.23^{a,b}	23.66 ± 0.03^{c}
Nitric oxide RAS	66.56 ± 0.48^{h}	56.03 ± 0.16^{g}	36.51 ± 0.09^{d}	24.82 ± 0.08^{c}	37.91 ± 0.36^{e}	43.09 ± 0.39^{f}	21.01 ± 0.17^{c}	17.67 ± 0.07^{a}	19.09 ± 0.00^{b}
Super oxide RAS	70.80 ± 0.07^{h}	41.01 ± 0.02^{f}	34.55 ± 0.02^{e}	19.50 ± 0.31^{a}	28.25 ± 0.37^{b,c}	51.13 ± 0.37^{g}	34.50 ± 0.04^{c}	28.99 ± 0.09^{c,b}	30.38 ± 0.10^{d}
Reducing power activity	755.5 ± 1.12^{h}	354.5 ± 0.55^{g}	187.3 ± 1.71^{e}	138.5 ± 1.52^{c}	172.1 ± 1.55^{d}	309.9 ± 1.76^{f}	101.87 ± 0.00^{a}	118.4 ± 0.62^{a}	135.5 ± 1.51^{b}

AA-ascorbic acid, BHA-butylated hydroxyl anisole.

* The values are mean of triplicates with (±) standard deviation (mean ± S.D. n = 3). Different superscript letters (a–h) in rows indicate significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

3.4. GC–MS analysis

A total of 10 major compounds was detected in the GC–MS analysis of *Ipomoea pes-caprae* acetone extract. The detected compounds RT, name, and structure and chromatogram are given in supplementary Fig. 3 and Supplementary Table 1. 2-Phenyl-4*H*-chromen-4-one was identified as a predominant compound with a retention time of 14.08 min, followed by 10-octadecenoic acid with a retention time of 18.40 min. Remaining secondary metabolites detected in the acetone extract were present in the least quantities and belong to fatty acids, flavones, nitrogen-containing compounds, carboxylic acids, terpenes, cyclic and bicyclic ketones. A total of 8 and 5 compounds were detected in LAF2 and 46HA fractions respectively (Supplementary Table 2 and 3). Supplementary Fig. 4 shows the GC–MS chromatogram of LAF2 and 46HA fractions. These phytocompounds might be responsible for various pharmacological properties of *Ipomoea pes-caprae*.

3.5. Structural elucidation of the isolated compound

The structure of the isolated compound ($C_{15}H_{10}O_4$, yellow power, 225–227 °C) was confirmed using various spectral study data, such as UV, FT-IR, LC—MS, ^{1}H, ^{13}C, DEFT-135, HMBC, and HSQC-NMR. The

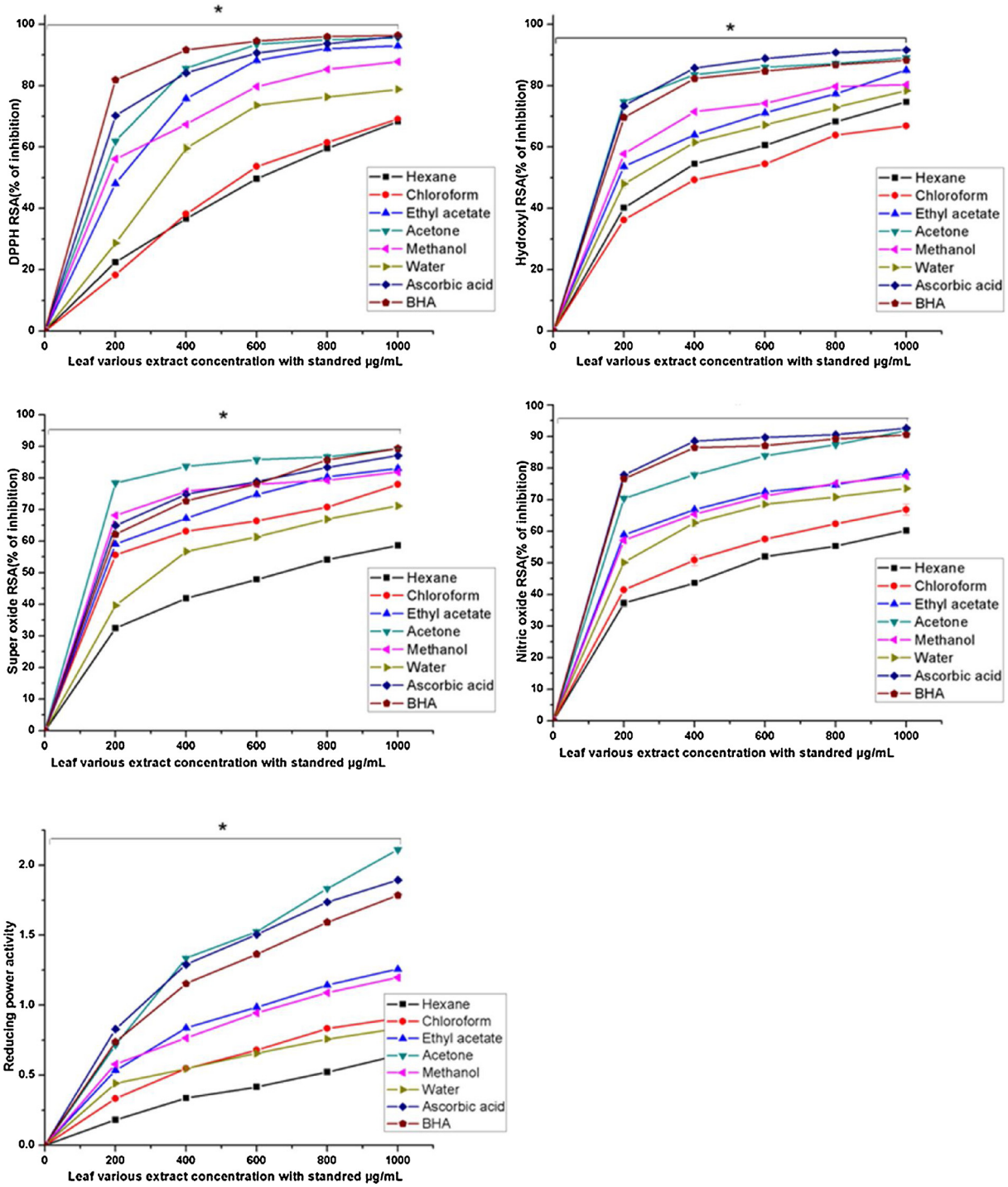

Fig. 2. Antioxidant potential of various extracts of *Ipomoea pes-caprae*.

UV–vis spectrum (Supplementary Fig. 5) of the isolated compound exhibited two absorbance maxima at λ_{max} 283 nm and λ_{max} 337 nm which correspond to $\pi \rightarrow \pi^*$ (C═C, C═O) and $n \rightarrow \pi^*$ for α, β unsaturated C═O group respectively.

The FT-IR spectrum of the isolated compound shows a broad absorption band at 3396 (s) cm^{-1} which indicated the presence of hydroxyl groups. The absorption bands at 1675 (m) and 1592 (w) cm^{-1} are characteristic for the occurrence of aromatic −C═C− groups. The absorption bands at 948 (s), 828 (s) and 690 (5) cm^{-1} indicated the presence of −C−H group out of plane bending of the aromatic compounds and also absorbed aliphatic −C−H bend at 1375 (m) and 1468 (m). The bands at 1235 (s), 1146 (s) and 1060 (s) cm^{-1} show the presence of −C−O− bending mode. The occurrence of the C−O−C group was confirmed by the bands at 543 (s) and 430 (m) cm^{-1}. The FT-IR spectrum (Supplementary Fig. 6) results are in good agreement with a coumarin compound functional groups.

LC-ESI-MS m/z (% intensity): 254.96. The mass accuracy results for LC–MS of the isolated compound (Supplementary Fig. 7) were sample run in the positive mode. ^{1}H-NMR (500 MHz, DMSO-d_6): δ_H 5.74 (1H, s, H-3), δ_H 10.13 (1H, s, OH-5), δ_H 6.16 (1H, d, J = 2 Hz, H-6), δ_H 10.42 (1H, s, OH-7), δ_H 6.27 (1H, d, J = 2 Hz, H-8), δ_H 7.31–7.36 (5H, m, H-2′-6′) (Supplementary Fig 8). ^{13}C-NMR (500 MHz, DMSO-d_6): δ_C 162.19 (C-2), δ_C 110.62 (C-3), δ_C 156.49 (C-4), δ_C 160.39 (C-5), δ_C 99.62 (C-6), δ_C 157.23 (C-7), δ_C 95.13 (C-8), δ_C 157.57 (C-9), δ_C 101.06 (C-10), δ_C

Table 5
Antibacterial activity of various solvent leaves extracts and isolated compound of *Ipomoea pes-caprae*.

S. No.	Organisms	Diameter of the zone of inhibition (in mm)*							
		Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water	Isolated compound	Positive control#
2	*Klebsiella pneumoniae*	10.67 ± 0.58^{b,c}	9.20 ± 0.26^{b}	10.00 ± 0.00^{a}	10.33 ± 0.58^{a}	10.13 ± 0.15^{d}	00.00 ± 0.00^{a}	12.04 ± 0.45^{b}	22.67 ± 0.29^{c}
3	*Salmonella typhi*	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}	11.67 ± 0.58^{c}	12.00 ± 0.00^{c}	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}	12.19 ± 0.21^{b,c}	29.23 ± 0.03^{d}
4	*Shigella flexneri*	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}	13.03 ± 0.06^{e}	11.10 ± 0.10^{b}	09.00 ± 0.00^{c}	00.00 ± 0.00^{a}	15.00 ± 0.76^{e}	21.50 ± 0.87^{b}
5	*Staphylococcus epidermidis*	10.67 ± 0.58^{b,c}	09.67 ± 0.58^{b,c}	12.33 ± 0.58^{d}	10.33 ± 0.58^{a}	09.00 ± 0.00^{c}	00.00 ± 0.00^{a}	10.11 ± 0.00^{a}	30.29 ± 0.03^{e}
6	*Streptococcus pneumoniae*	15.17 ± 0.29^{d}	10.07 ± 0.12^{d}	11.67 ± 0.58^{c}	12.00 ± 0.00^{c}	09.07 ± 0.06^{c}	00.00 ± 0.00^{a}	12.51 ± 0.32^{c,d}	22.33 ± 0.31^{c}

* The values are mean of triplicates with (±) standard deviation (mean ± S.D. n = 3). Different superscript letters (a–f) in column indicate significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

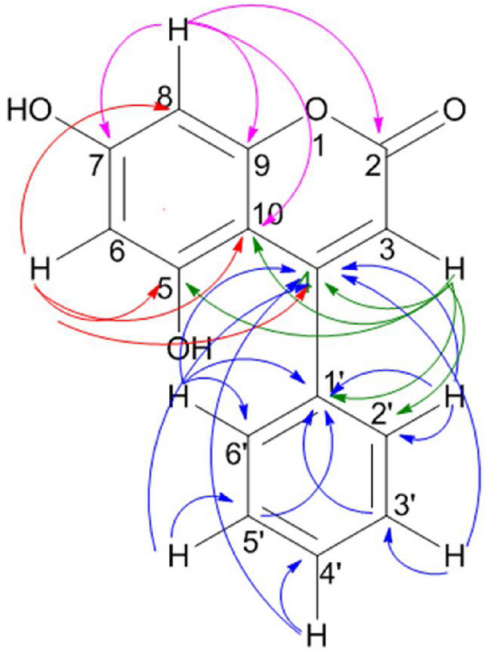

Fig. 3. HMBC correlation of isolated compound.

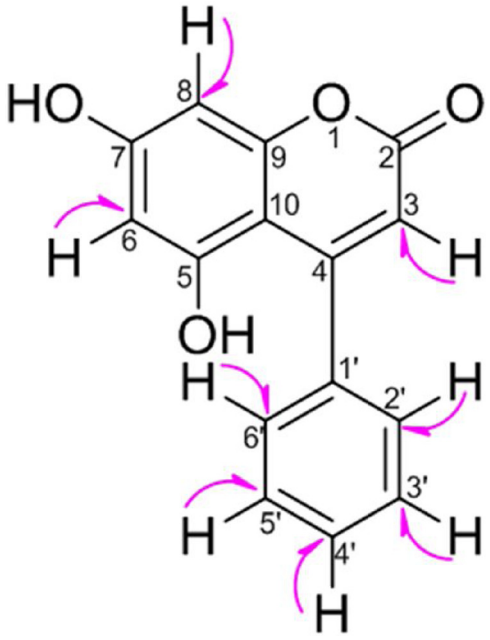

Fig. 4. HSQC correlation of isolated compound.

140.04 (C-1′), δ_C 127.85 (C-2′), δ_C 127.71 (C-3′), δ_C 128.23 (C-4′), δ_C 127.71 (C-5′), δ_C 127.85 (C-6′) (Supplementary Fig 9).

The results of HMBC spectroscopy shows the coupling correlation of H-3 (δ_H5.74) with C-4 (δ_C 156.78), C-5 (δ_C 160.40), C-10 (δ_C 101.04), C-1′ (δ_C 140.62) and C-2′ (δ_C 127.58); H-6 (δ_H 6.16) with C-4 (δ_C 156.53), C-5 (δ_C 160.70), C-8 (δ_C 95.08) and C-10 (δ_C 101.33); H-8 (δ_H 6.27) with C-2 (δ_C 162.02), C-7 (δ_C 157.85), C-9 (δ_C 157.04) and C-10 (δ_C 101.33); H-2′ (δ_H 7.31 with C-1′ (δ_C 140.12), C-2′ (δ_C 127.66) and C-4 (δ_C 156.86); H-3′ (δ_H7.33) with C-1′ (δ_C 140.12), C-3′ (δ_C 127.66) and C-4 (δ_C 156.86); H-4′ (δ_H7.36) with C-4′ (δ_C 127.66) and C-4 (δ_C156.86); H-5′ (δ_H7.33) with C-1′ (δ_C 140.12), C-5′ (δ_C 127.66) and C-4 (δ_C 156.86); H-6′ (δ_H 7.31) with C-1′ (δ_C 140.12), C-6′- (δ_C 127.66) and C-4 (δ_C 156.86) (Supplementary Fig 10). These correlations confirmed the long range couplings of the isolated compound (5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one) (Fig. 3).

The results of HSQC spectroscopy revealed that the aromatic protons are coupled with corresponding carbons (Supplementary Fig 11). The H-3 proton (δ_H 5.74) was coupled with C-3 (δ_C 110.74); H-6 (δ_H 6.16) with C-6 (δ_C 99.35); H-8 (δ_H 6.26) with C-8 (δ_C 95.32) and H-2′-H-6′ (δ_H 7.31) with C-2′ (δ_C 127.23) and C-6′ (δ_C 127.85) respectively (Fig. 4). The DEPT-135 spectrum (CH, CH_3 positive and CH_2 negative) results indicated the presence of 8 CH groups due to the appearance of 8 carbons in the positive mode such as δ_C 110.62 (C-3), δ_C 99.61 (C-6), δ_C 95.13 (C-8), δ_C 127.85 (C-2′), δ_C 127.71 (C-3′), δ_C 128.23 (C-4′), δ_C 127.38 (C-5′), δ_C 127.82 (C-6′) which indicate that the isolated compounds have 8 CH groups (Supplementary Fig 12). Based on the spectral data the isolated compound was confirmed as 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one (a coumarin compound). This is the first-hand report on the isolation and identification of 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one in *Ipomoea pes-caprae* extract.

3.6. Biological activities of the isolated compound

The isolated compound 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one harbor remarkable dose-dependent antiradical ability (Fig. 5). 5,7-Dihydroxy-4-phenyl-2*H*-chromen-2-one expressed significant-high antiradical activity on DPPH radicals with lowest IC_{50} values (08.15 ± 0.15 µg/mL) followed by hydroxyl radicals (IC_{50} values 14.11 ± 0.05 µg/mL) (Table 4). The least activity was found in the ferric reducing power assay with high IC_{50} values (101.87 ± 0.00 µg/mL). The results of antibacterial activity revealed that the isolated compound has significant antibacterial potential against most of the tested pathogens and the maximum growth inhibition was observed against *Escherichia coli* (26.25 ± 0.10) followed by *Shigella flexneri* (15.00 ± 0.76) (Table 5). The treatment of 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one decreased the HCT-116 human colon cancer cell viability upto 30% after 24 h of treatment at 50 µM concentrations with lowest IC_{50} values (14.11 ± 0.05 µg/mL) (Fig. 6). The isolated coumarin compound displayed strong interactions with the angiopoietin-2 receptor (PDB: 1Z3S) (Fig. 7). 5,7-Dihydroxy-4-phenyl-2*H*-chromen-2-

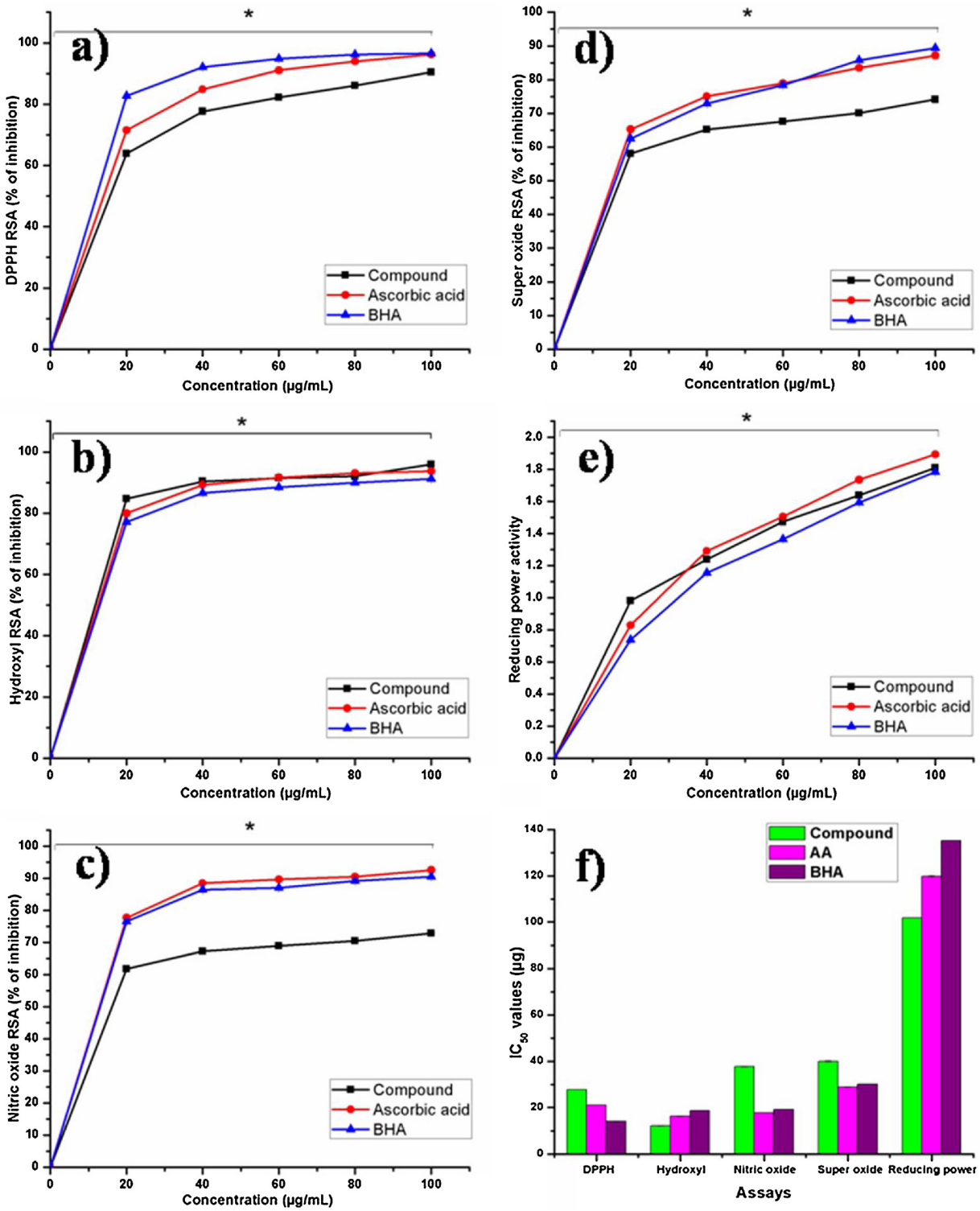

Fig. 5. Antioxidant activities of isolated compound. a) DPPH radical scavenging activity (RSA), b) Hydroxyl RSA, c) Nitric oxide RSA, d) Super oxide RSA, e) Reducing power activity, f) IC_{50} value of various assays.

one showed 2NH…O type of H-bond interactions with LYS310 and GLY347 at a distance of 2.41 Å and 3.11 Å respectively, along with other non-bonded interactions and other residues lining the active site pocket. The docking score and binding energy are –9.61 and –6.92 kcal/Mol respectively (Table 6).

4. Discussion

Previous reports revealed that the *Ipomoea pes-caprae* leaf methanol [21] and ethyl acetate extracts [36] contain various classes of phytochemicals such as tannins, saponins, flavonoids, alkaloids, glycosides, and phenolic compounds, unsaturated steroids, and triterpenes. The diversification in phytochemicals of extracts due to the difference of solvent polarity. Kumar et al. [37] observed the maximum phenolic

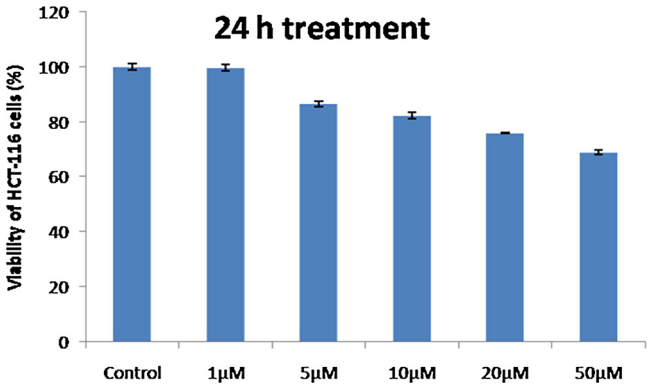

Fig. 6. Anti-proliferative effect of isolate compound on HCT-116 human colon cancer cells.

content (20.02 mg of GAE/g dry weight) in the methanol extract among the tested four different extracts (hexane, dichloromethane, ethyl acetate, and methanol) of *Ipomoea pes-caprae*. Similarly, Parekh et al. [38] also observed higher polyphenols (880 ± 1.53 mg of GAE/g dry weight) and flavonoids (128.78 ± 0.23 mg of GAE/g dry weight) content in the methanol extract of *Ipomoea pes-caprae* leaves. The quantitative phytochemicals estimation results revealed that the *Ipomoea pes-caprae* leaves acetone extract contains high amount of both primary and secondary metabolites which is in contrary to the previous reports [39,40]. Variations in the total quantity of secondary metabolites due to the difference in agro ecological factors of the plant source, extraction techniques, and analysis methods [41]. Moreover, present study concludes that the sequential extraction method is suitable and good for extraction of a higher amount of secondary metabolites. A limited number of reports were available on the radical scavenging effect of different solvent extracts of *Ipomoea pes-caprae* [21,40,41] that supported the present results. However, the findings of the present study are quite similar to other species of *Ipomoea* genus [38].

Bragadeeswaran et al. [42] studied the antibacterial activity of different solvent (methanol, acetone, chloroform, and *n*-butanol) leaf extracts of *Ipomoea pes-caprae* which results revealed that the methanol extract harbors maximum growth inhibition potential against *E.coli* (20 mm), followed by acetone extract (15 mm). Similar findings were reported by Kumar et al. [38] who studied the antimicrobial potential of different solvent (hexane, dichloromethane, ethyl acetate, and methanol) extracts of *Ipomoea pes-caprae*. The present antibacterial results are superior to the aforementioned reports and the maximum growth inhibition potential was noted in the mid-polar (acetone) solvent extract. Moreover, the present findings are in good agreement with the reports of Nagababu and Umamaheswara Rao, [43] who studied the antibacterial potential of whole *Ipomoea pes-caprae* acetone extract against various pathogens. Thus, the present study revealed that the sequential extraction method may facilitate the extraction of various active phytochemicals from *Ipomoea pes-caprae*.

Recently, twenty compounds were identified from the hexane extract of leaves and stem of *Ipomoea pes-caprae* and those belong to sesquiterpene, an aliphatic alcohol, diterpene alcohol, vitamins, and phytosterol [44]. An earlier study documented the isolation and identification of 19 compounds in the methanol extract of *Ipomoea pes-caprae* which belongs to flavones, cyclic ketones, and steroidal compounds [38]. The results of present GC–MS also harbor similar kinds of phytochemicals and these phytocompounds are known to be exhibited anticancer, antioxidant and antibacterial activity that supported the present outcome [38,44]. Previously, a few coumarin compounds were isolated from *Ipomoea* genera such as 5-hydroxy-7-methoxy coumarin and scopoletin [45–47]. Bioassay-guided isolation method lead to isolation of many high potent antioxidant compounds such as 3,4-dihydroxy phenyl-*O*-β-D-glucoside, gypenoside XLVI, gypenoside L, ginsenoside 3,4-dihydroxy phenyl-*O*-β-D-glucoside, 7-*O*-galloylcatechins, catechins and methyl gallate, lyoniresinol-3α-*O*-β-arabinopyranoside, lyoniresinol-3α-*O*-β-rhamnoside, and afzelechin-3-*O*-L-rhamno-pyranoside, apigenin, ferulic acid, vitexin, caprolactam, rosmarinic acid, and globoidnan A, 5-hydroxy-3′, 4′, 7-trimethoxyflavone, and quercetin-3-O-α-L-rhamnoside(1→6)-β-D-glucose from various plant sources like *Gynostemma pentaphyllum* [48], *Acacia hydaspica* [49], *Rhizophora apiculata* [50] *Origanum rotundifolium* [51], *Lippia nodiflora* [52] and *Memecylon edule* [53] which strengthen the present investigation. Most of the antioxidant compounds stated above are phenolic, flavonoid and flavone in nature and possess vital biological activities like anticancer and antimicrobial which supported the present findings.

This is the first report of antimicrobial, antioxidant and anti-proliferative activities of isolated compound 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one. Therefore, no previous data are available to compare its tested biological potential. Generally, coumarin nature compounds have significant therapeutic potential and possessing a wide spectrum of biological activities including antioxidant, antibacterial, antifungal, anti-inflammatory and antitumor activities [54]. Coumarins are a typical phenolic compound, which contains free hydroxyl groups of 6-, 7- or 8- position that responsible for their vital biological activities like antiradical and antibacterial capacity [55,56] that supported the present outcome. The literature stated that the biological activities of coumarins and polyphenols depend on their number of free OH group and its position [55]. Yasameen et al. [54] reported that the

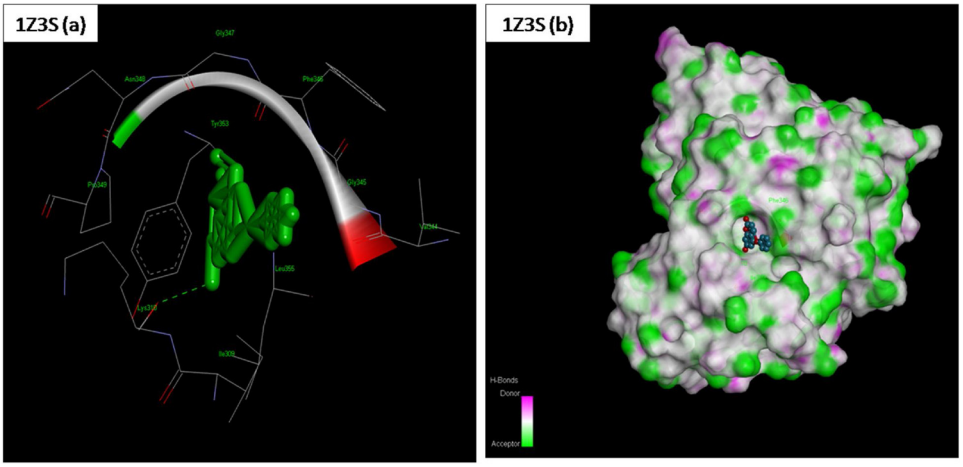

Fig. 7. a) Interactions of 5,7-Dihydroxy-4-phenyl-2*H*-chromen-2-one with the active site residues of 1Z3S, b) Surface diagram of 5,7-Dihydroxy-4-phenyl-2*H*-chromen-2-one binding at the active site of 1Z3S.

Table 6
Docking results (kcal/mol) of 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one with angiopoietin-2 receptor (1Z3S).

Binding energy (ΔG)	Ligand efficiency	Inhibit constant (Ki) μM	Electrostatic energy	Intermolecular energy	vdW + Hbond + desolve energy	Final total internal energy	Torsional free energy	Unbound system's energy	No. of H bond	H-interactions	H bond distance (Å)
−6.92	−0.36	8.46	0.03	−9.61	−9.64	27.34	2.68	27.34	2	LIG1:O - A:LYS310:O LIG1:O - A:GLY347:O	2.41 3.11

osthenol a coumarin compound which contains a free OH group at C7 and prenylation at C8 position have good antibacterial potential against Gram-positive bacteria. Moreover, the previous report suggested that C7-free hydroxyl group is essential for the growth inhibition of Gram-positive bacteria [57]. Whereas, the present isolated compound shown remarkable growth inhibition on Gram-negative bacteria followed by Gram-positive bacteria and the reason might be the position (at C5 and C7) of free hydroxyl groups.

Similarly, several coumarin nature compounds such as osthole, fraxin, and grandivittin have inhibited the proliferation [58,59], migration and invasion of various types of cancer cells [60] which is in good agreement with the present outcome. The National Cancer Institute guidelines indicated that an isolated compound possesses $IC_{50} \leq 4\,\mu g/mL$ considered as an active compound. According to the NCI guidelines 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one has quite higher IC_{50} value. Similar findings were documented by Fakai et al. [61] who isolated 2 coumarins from *Ruta angustifolia* that show sustainable antiproliferation potential on HCT-116 cells, however, their IC_{50} values are higher than National Cancer Institute guidelines which supported the present results. Doxorubicin a commercial anticancer possess significant low IC_{50} value on HCT-116 cells [61] than 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one, however, 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one may be serve as molecular templates to develop highly potent anticancer agent in drug development.

Coumarin compounds are well documented for their anti-proliferative potential of various types of cancer cells and inhibition of angiogenesis [62]. Angiogenesis is one of the important targets of coumarin derivatives, which prevent the angiogenesis by inhibiting proliferation, migration, and tubule formation. Coumarins also have known to inhibit kinases and metalloproteins that resulted in inhibition of cell growth (anti-proliferative activity). Angiopoietin-2 is a metalloprotein, act as a kinase and with vascular endothelial growth factor facilitates endothelial cell migration and proliferation in angiogenesis [63]. Angiopoietin signaling directly corresponds with angiogenesis, which proceeds to sprout, endothelial cell migration, proliferation, and vessel destabilization and stabilization [64]. Angiogenesis is a vital process in the growth and development of granulation tissue. However, it is an initial step in the transition of tumor formation, thus leading to the use of angiogenesis inhibitors in the treatment of cancer [65]. Particularly, the reduced level of angiopoietin-2 promotes cell death and disrupts vascularization [66]. Angiopoietin-2 in the absence of vascular endothelial growth factor leads to endothelial cell death and vascular regression [67]. Thus, many researchers are targeting angiopoietin-2 to reduce tumor angiogenesis and delay the growth of tumor cells. Several findings highlighted the therapeutic value of angiopoietin-2 inhibitors and some of the angiopoietin-2 inhibitors are already in clinical trials [68]. Therefore, the present study also targeted the angiopoietin-2 active site with 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one. The previous report indicated that the 5,7-dihydroxy-4-phenyl coumarin is a good molecular template in the development new drugs [69] that strengthen the present investigation. The overall findings of the present investigation revealed that isolated 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one harbor significant antioxidant, antibacterial, and anticancer potential and may inhibits the activity of angiopoietin-2. However, the present findings have some limitations such as solubility and yield of the isolated compound. Hence, we plan to design and synthesis a novel molecule with new functional groups to increase biological potential and counteract the limitations in future.

5. Conclusions

The leaves of *Ipomoea pes-caprea* is a good source of a variety of phytoconstituents especially, phenolic compounds and flavonoids. The acetone extract of *Ipomoea pes-caprea* leaves exhibited strong antioxidant and antibacterial activity. DPPH radical scavenging activity guided purification resulted in the isolation of an antioxidant coumarin

compound. The results of spectral data like UV, FT-IR, LC–MS, ^{1}H and ^{13}C-NMR, HMBC, HSQC, and DEPT-135 confirmed that the isolated compound is 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one. The isolated 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one possess significant antioxidant, antimicrobial and antiproliferative properties. 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one show good interaction with the active site of angiopoietin-2. The present study suggests that the 5,7-dihydroxy-4-phenyl-2*H*-chromen-2-one may be used as a potent bioactive compound in the development of plant-based antioxidant, antibacterial, and anticancer drugs in the future.

Conflict of interests

The authors declare that they have no conflict of interests to declare in this paper.

Authors' contributions

S.V. and M.K. designed the experiments. V.A. and S.R. performed the research work (equally contributed) and drafted the manuscript. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eujim.2019.100984.

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