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Isolation and characterization of undescribed flavonoid from *Abrus precatorius* L. based on HPTLC-DPPH bioautography and its cytotoxicity evaluation

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Abstract

Background Naturally derived compounds play a tremendous role as a drug as well as lead structure for the development of APIs. Therefore, isolation and characterization of compounds from nature are needed to alleviate life-threatening diseases. *A. precatorius* L. belongs to the family Leguminosae and is valued for its medicinal properties. Therefore, in this study, efforts are being made to isolate bioactive entity based on HPTLC-DPPH bioautography from APHA extract. Among all the separated compounds on TLC plate, the one (APSP-3) at $R_f = 0.67$ showed significant antioxidant activity, and hence, APSP-3 was further subjected to isolation, purification, and structural characterization using diverse analytical modus operandi such as 1D and 2D NMR, FTIR, HPLC-MS/MS, and elemental analysis. In addition, antioxidant and cytotoxicity evaluation of APHA extract and APSP-3 was pursued by standard DPPH and colorimetric MTT assays, respectively.

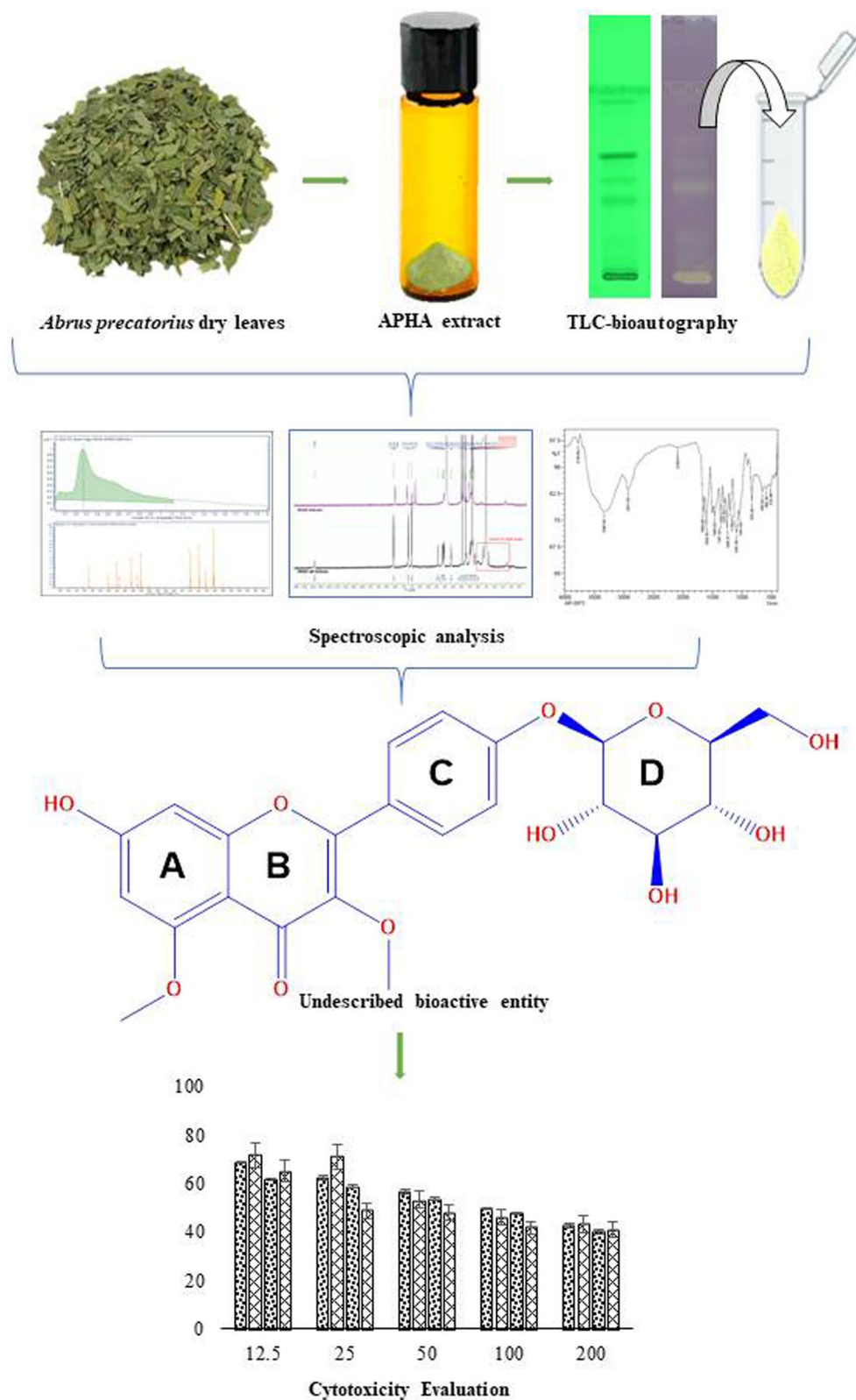
Results Antioxidative isolated compound APSP-3 was scrutinized based on HPTLC-DPPH bioautography. The APSP-3 was found novel and spectroscopic data revealed the plausible structure; 7-hydroxy-3,5-dimethoxy-2-(4-((3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) phenyl)-4H-chromen-4-one. Moreover, APSP-3 ascribed higher free radical scavenging activity with $IC_{50} = 38.70 \pm 3.5 \mu\text{g/mL}$ than standard ascorbic acid ($75.19 \pm 1.5 \mu\text{g/mL}$). Cytotoxicity evaluation of APHA extract exhibited IC_{50} value $122.09 \mu\text{g/mL}$ for HepG2, $122.61 \mu\text{g/mL}$ for MCF-7, and $48.08 \mu\text{g/mL}$ for HCT116 cell lines, while APSP-3 displayed IC_{50} values 96.75 for HepG2, 61.67 for MCF-7, and $47.61 \mu\text{g/mL}$ for HCT116 cell lines.

Conclusions In a nutshell, HPTLC-directed bioautography leads to the capturing of new flavonoid entity having antioxidant potency from APHA extract. The IC_{50} values obtained from cytotoxicity establish a dose-response relationship helping to determine the concentration at which a substance begins to exhibit toxic effects. This fundamental information is crucial for establishing safe dosage level in medical and pharmaceutical applications. Further, research engrossed in assessing other bioactivities involving in silico and in vivo studies obliged to offer a promising and secure portrayal for clinical implications.

Keywords *Abrus precatorius* L., HPTLC-DPPH bioautography, Isolation, Spectroscopic characterization, Antioxidant activity, Cytotoxicity

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Graphical abstract



Background

Since time immemorial, medicinal plants are an inexhaustible reservoir for traditional medicine, modern medicines, nutraceuticals, food supplements, and chemical entities for synthetic drugs [1]. In India, nutritional metrics and conventional plant therapies are ubiquitously used as supported by Ayurvedic and other indigenous systems of medicine [2]. Prior to high throughput scrutinizing and the post-genomic era, more than 80% of therapeutic compounds were either solely natural products or took their inspiration from compounds acquired from natural avenues. In the period 2005 to 2007, thirteen natural product-related medicines were authorized [3].

Each medicine is comprised of two primary elements. Active pharmaceutical ingredients (APIs) and an additional part called an excipient. API, the chemically and biologically active major ingredient in any medicine, can be either naturally derived from plants or synthetic [4]. A couple of commercial pure natural products developed from plant sources were introduced for therapeutic use and the first drug was morphine, found from chopped seed pods of *Papaver somniferum*. Consequently, other drugs invented from natural assets have indeed revolutionized medicine. Examples include antibiotics: penicillin, tetracycline, erythromycin, antimalarials: quinine, artemisinin, antiparasitic: avermectin, lipid control agents: lovastatin and analogs, immunosuppressant cyclosporine, rapamycin, and anticancer: paclitaxel, irinotecan [3].

Bioactive principles belong to secondary metabolites, work in conjunction with nutrients and fibers to serve as an illness-defense shield [5]. Among, flavonoids (flavan nucleus and a C₆–C₃–C₆ carbon skeleton) are one of the most bioactive remedies and have been used in traditional healthcare all over the world. Over 8000 different flavonoids have been recognized so far [6, 7], famed for bioactivities, and widely accepted as therapeutic agents [8, 9]. However, these polyphenols have found their utmost use in the realm of medicine. Flavonoids have been widely employed as anticancer, antibacterial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents. A plethora of flavonoids has been isolated, and this number keeps rising. Some examples of isolated bioactive flavonoids are hesperidin and aurone (anticancer), quercetin, and hesperidin (antioxidant) [9]. Additionally, concern about the adverse consequences of synthetic products is growing. Some synthetically manufactured antioxidants, such as dibutylhydroxytoluene and butylated hydroxyanisole, have been shown to promote carcinogenic activity [10–12], whereas naturally retrieved phenolic antioxidants have been shown to manifest a variety of health-promoting effects [12]. Moreover, the tendency

of substituting natural antioxidants for synthetic antioxidants is rising [13]. Plenty of research on antioxidants in relation to diverse flavonoids has also been conducted and concluded that flavonoids can be used as potential drugs to prevent oxidative stress [14–16]. Hence, this study is focused on the isolation and characterization of novel flavonoid entity with antioxidant potential.

The abundance of bioactive scaffolds in plants motivates the ongoing advancement of sorting methods and bioassays for isolation and identification [17]. Currently, thin layer chromatography-direct bioautography (TLC-DB) as an effect-directed analysis has steadily grown in popularity. The combine direct biological assays conducted on the adsorbent layer with separation, making it particularly valuable for identifying biologically active phytochemicals [17, 18].

In the context of the growing requisition of herbal medicines from the consumer, there is urgency to appraise the efficacy of products as well as thoroughly ensure their safety [19]. Therefore, a reliable, sensitive, and quantitative in vitro bioassays are needed to predict the cytotoxicity and drug response metric values of constituents to various tissues [20, 21]. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) scrutiny has become a fundamental gauge for monitoring cell viability and drug response metric values. The MTT assay is one of the most frequently employed colorimetric assay for determining the respiratory capacity of the mitochondrial succinate-tetrazolium reductase system, which uses Nicotinamide Adenine Dinucleotide Hydrogen (NADH) to convert yellow MTT to purple formazan product [21, 22]. Hence, in the present study, MTT assay is adopted to understand pharmacology metrics such as IC₅₀ (the inhibition concentration of a compound where the response is reduced by 50% and to describe potency of novice component).

Abrus precatorius L. (*A. precatorius*) is a woody twining plant belonging to Abrus genus (family; Leguminosae) known as rosary pea and jequirity pea, found in tropical and subtropical regions such as China, India and Nigeria [23]. In Ayurveda, *A. precatorius* leaves are used as a laxative, expectorant and aphrodisiac medicine. This plant is also employed in urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas/leukemia, and dysmenorrhea conditions [24] and it also demonstrates a range of pharmacological activities [23, 25, 26]. It is also a major ingredient of many marketed polyherbal digestive mixtures such as Chatak® [27]. These medicinal utilities have been attributed to various phytoconstituents, belonging to the chemical family of alkaloids, steroids, flavonoids, and polyphenols. Several compounds like abrine, trigonelline, abruslactone A, hemiphloin, abrusoside A, arabinose, galactose, xylose,

choline, hypaphorine, precatorine glycyrrhizin, montanyl alcohol, inositol, D-monomethyl ether, pinitol, etc., have been identified [24].

In our investigation, a bioactive flavonoid (APSP-3) from the leaf of *A. precatorius* hydroalcoholic (APHA) extract was obtained based on high-throughput and cost-effective high-performance thin layer chromatography-1,1-diphenyl-2-picrylhydrazyl (HPTLC-DPPH) bioautography. Moreover, the structure of APSP-3 was elucidated using 1D (^1H , ^{13}C), 2D NMR spectrum, FTIR, HPLC-MS/MS, and elemental analysis. Further, to stamp out the safety and efficacy of the isolated flavonoid, a cytotoxicity assessment was done using colorimetric analysis.

Methods

Plant collection and authentication

The fresh leaves of *A. precatorius* were collected from Ahmedabad district, Gujarat, India, in December 2021. The plant was authenticated by a taxonomist, and a voucher specimen was deposited with reference identity at the Department of Botany. The leaves were treated, and the extract was prepared as mentioned in our earlier publication [25].

Preparation of plant extract

The leaves were thoroughly washed twice with distilled water (DW) to ease dust and soil particles. Further, the leaves were dried in the shade for 30 days, pulverized with a mechanical grinder to obtain a fine powder, and then stored at 4 °C for downstream analysis. For extraction, 10 g of leaf powder was mixed with 100 mL hydroalcohol (methanol:DW; 1:1) and extracted by maceration method to obtain *A. precatorius* hydroalcoholic (APHA) extract. Further, extract was filtered through Whatman No.1 filter paper and collected in a Petri dish. The filtrate was dried under reduced pressure in a rotary evaporator for the efficient and gentle removal of solvents at 40–45 °C. The yield of extract was stored in an air-tight amber bottles at 4 °C to avoid direct light and excessive heat [25].

Instrumentation

Phytochemical screening was carried out using High-Performance Thin Layer Chromatography (HPTLC) (CAMAG) system equipped with visionCATS software (version 2.5; S/N 18262.1). High-Performance Liquid Chromatography-tandem Mass Spectrometry (HPLC-MS/MS) (1290 Agilent Infinity II 1300 bar and 6470 QQQ) was accomplished to check the purity and exact mass of the compound. Fourier-Transform Infrared spectroscopy (FTIR; Bruker Alpha, model 8400) spectra were carried out with potassium bromide (KBr) pellets within

400–4000 cm^{-1} region at room temperature, using the KBr press pellet method to get information on the existence of chemical functional groups. Chemical shifts for ^1H nuclear magnetic resonance (NMR; 400 MHz) spectra of the compound were recorded in deuterated dimethyl sulfoxide ($\text{DMSO}-d_6$) and methanol (MeOD) solvents for clear elucidation, while chemical shift for ^{13}C NMR (101 MHz) spectra was performed in deuterated DMSO solvent with tetramethylsilane (TMS) as an internal standard on Bruker ULTRASHIELD 400 Advance III (Bruker Biospin, Switzerland). 2D NMR including Heteronuclear Multiple Quantum Coherence (HMQC) spectrum, Heteronuclear Multiple-Bond Coherence (HMBC) spectrum, Correlated Spectroscopy (COSY), Distortionless Enhancement by Polarization Transfer-135 (DEPT-135), and Nuclear Overhauser Effect Spectroscopy (NOESY) were also recorded to explain the structure of an isolated flavonoid (APSP-3). Elemental analysis was carried out using a CHNS/O analyzer (Unicube).

Preparation of sample

The crude APHA extract (10 mg) was weighed and dissolved into 1 mL of hydroalcohol, sonicated for 10 min for complete dissolution, and used as a sample for flavonoids fingerprinting.

High-performance thin layer chromatography (HPTLC) conditions for flavonoids fingerprinting

HPTLC was performed on a 5×10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F₂₅₄ (Merck, Germany). A sample (200 $\mu\text{g}/\text{spot}$) of 8 mm length was applied by the Hamilton microsyringe (Switzerland) with the nitrogen flow (150 nL/s) at 25 ± 2 °C. The sample was applied using a semi-automated Linomat 5 sample applicator (S/N 180344). The plate was developed up to 70% in an ascending manner in twin through chamber (10×10 cm) previously saturated (30 min) with the optimized mobile phase chloroform-methanol-DW (8:2:0.2, v/v/v). Subsequently, the TLC plate was air-dried and scanning was executed using TLC Scanner 4 (S/N 180404) to develop chromatograms at 254 and 366 nm. Densitograms and R_f values were also documented using the software [25].

Identification of bioactive compounds by HPTLC-DPPH bioautography

The APHA extract was dissolved in hydroalcohol and tested against DPPH using the HPTLC-DPPH bioautography assay. Using the TLC sprayer, the developed chromatoplate was sprayed with 0.2% methanolic DPPH solution. After spraying, the plate was kept in the dark

for 30 min, and then, observation was carried out. Yellow spots on a purple background show the antioxidant activity of the separated fraction [17].

Isolation and purification of APSP-3 from *A. precatorius* leaf extract

APHA extract (5.6 g) obtained was bound with 14 g silica gel (100–200 mesh size) to form an admixture and chromatographed over a silica gel column (500 mm length $\times$ 20 mm diameter). Successive elution was done in the following sequence, chloroform (120 mL) $\rightarrow$ chloroform–methanol (200 mL; 98:2, v/v) $\rightarrow$ chloroform–methanol (200 mL; 95:5, v/v) $\rightarrow$ chloroform–methanol (400 mL; 90:10, v/v) $\rightarrow$ chloroform–methanol (350 mL; 80:20, v/v). The fractions (12–24) collected were combined and re-chromatographed, and the solvent evaporated to gather a pure compound (230 mg).

High-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS)

The HPLC–MS/MS analysis was performed using a suitable system, such as the Triple TOFTM 5600 equipped with an electrospray ionization (ESI) source. The samples were prepared and analyzed in negative/positive ion modes, with the following parameters: nebulizer gas (Gas 1) of 45 psi; heater gas (Gas 2) of 45 psi; curtain gas of 35 psi; ion spray voltage of 7 eV; turbo spray temperature (TEM) of 300 °C; declustering potential (DP) of 60 V for MS; declustering potential (DP) of 100 V for MS/MS; collision energy (CE) of 10 for MS; collision energy (CE) of 35; and collision energy spread (CES) of 15 for MS/MS. The identification of compounds was carried out by comparing retention time and m/z values obtained by MS and MS/MS using PeakView software, while peak area values were determined by utilizing the extracted ion chromatograms (XICs) generated by the XIC manager in PeakView software. The application of these optimized parameters was crucial for the accurate identification and quantification of bioactive compounds in complex matrices, thereby improving the reliability and reproducibility of the experimental results [28, 29].

1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging efficacy of APHA extract and APSP-3 was assessed in terms of hydrogen donating potential using the steady 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by Blois method with minute modification [30]. In brief, 500 μ L (0.1 mM) of methanolic DPPH solution was assorted with various concentrations (12.5–200 μ g/mL) of APHA extract and APSP-3

compound. The resulting mixture was agitated vigorously and allowed to stand in dark for 30 min at 25 °C. Control was without the sample and methanol was used as a blank. The absorbance was measured at 517 nm using an EPOCH microplate spectrophotometer (BioTek Gen⁵™ software). Results were compared with standard ascorbic acid. The ability of DPPH radical scavenging activity was calculated by using the formula $[(A_0 - A_1)/A_0 \times 100]$ where A_0 is the absorbance of the control and A_1 is the absorbance of the APSP-3 solution.

Cytotoxicity assessment using

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

Cytotoxicity of APHA extract and APSP-3 was ascertained using the standard colorimetric MTT assay as described by Mosmann in 1983 [31]. The cell lines HepG2, MCF-7, and HCT116 were obtained from the National Centre for Cell Sciences (NCCS), Pune, India. The cells were cultured in 90% Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C until the confluency was achieved.

The cells were seeded at a density of $\sim 1 \times 10^4$ per well and incubated at 37 °C with 5% CO₂ for 24 h. After incubation media was removed and various concentrations of APHA extract and APSP-3 (12.5–200 μ g/mL) were added into the well. After 28 h of incubation, 10 μ L of MTT reagent (5 mg/mL PBS) was added and mixtures were re-incubated for 4 h by covering with aluminum foil. Ultimately, the remaining MTT solution was eliminated, and the produced formazan was dissolved in 100 μ L DMSO and incubated for 40 min in a CO₂ incubator. The absorbance of formazan was measured at 570 nm. The results were expressed as % cell viability of triplicate and IC₅₀ values were also obtained using different concentrations.

Results

Identification of bioactive compounds by HPTLC–DPPH bioautography

The APHA extract was first evaluated for DPPH hunting capacity by the conventional spectrophotometric method [25] trailed with the TLC–DPPH bioautography method to point out fractions having antioxidant potential. TLC–DPPH bioautography reveals three potential antioxidative fractions (Fig. 1). Among the uppermost (APSP-3) fraction was isolated, purified in sufficient quantity by column chromatography and further subjected to spectroscopic examination for structural elucidation by means of various spectroscopic analyses.

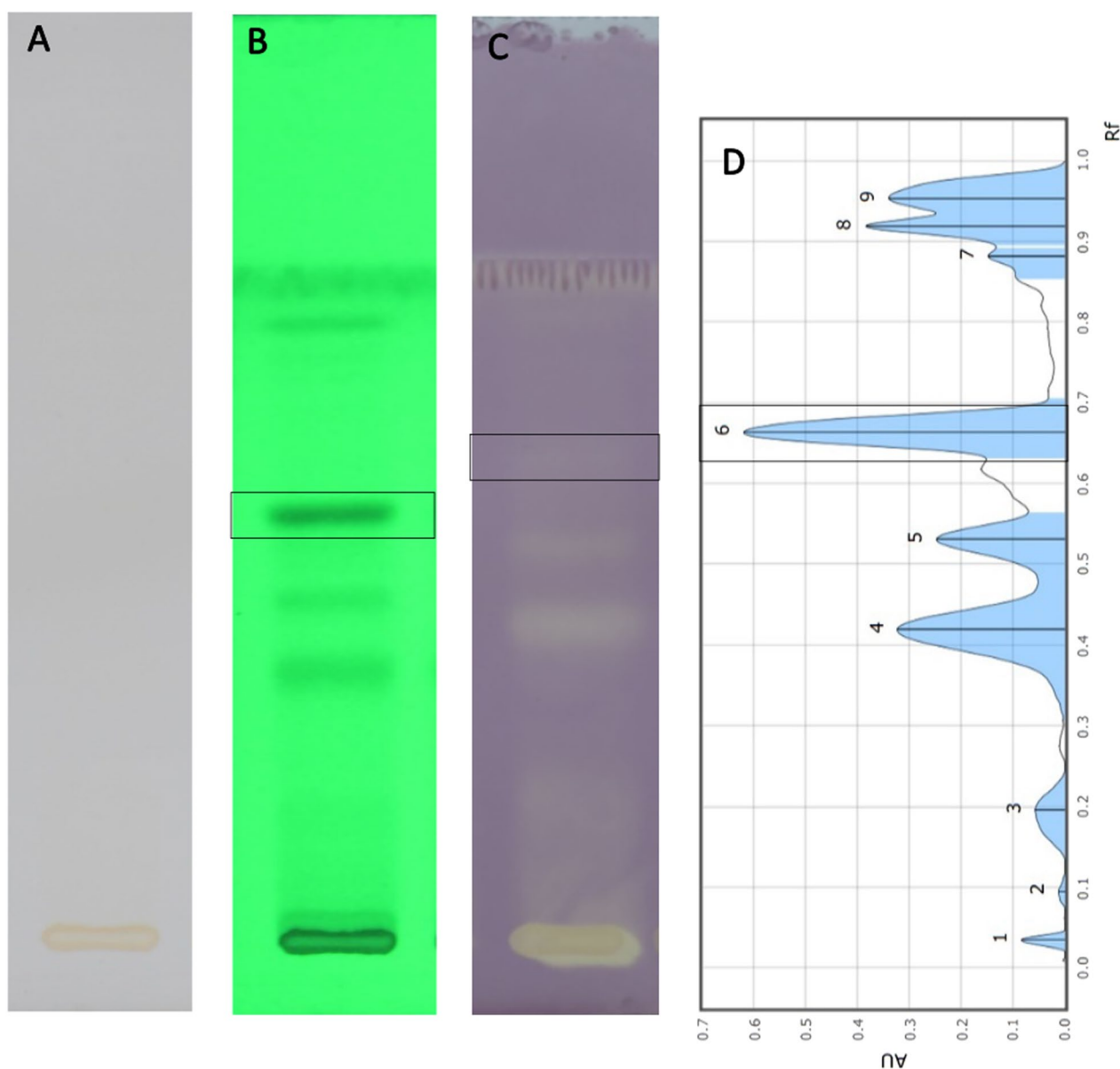


Fig. 1 TLC plate at **A** visible light **B** UV-254 **C** TLC-DPPH bioautogram **D** densitogram at 254 nm

Spectral characterization

The compound APSP-3 was acquired in the form of a light yellowish amorphous powder with a melting point of 124.5 °C. The results obtained from different spectral analysis are given below: Analysis found for (APSP-3; $C_{23}H_{24}O_{11}$): C=51.96; H=5.43; O=38.86. 1H NMR (400 MHz, $DMSO-d_6$) δ 12.88 (s, 1H), 8.11–8.09 (dd, 2H), 7.23–7.20 (dd, 2H), 7.01 (s, 1H), 7.00 (s, 1H), 5.42–5.41 (d, 1H), 5.17–5.16 (d, 1H), 5.10–5.09 (d, 1H), 5.07–5.05 (d, 1H), 4.63–4.61 (t, 1H), 3.95 (s, 3H), 3.75 (s, 3H), 3.49–3.40 (m, 3H), 3.30–3.19 (m, 3H). ^{13}C NMR (101 MHz, $DMSO-d_6$) δ 182.298, 163.378, 160.345, 158.713, 152.667,

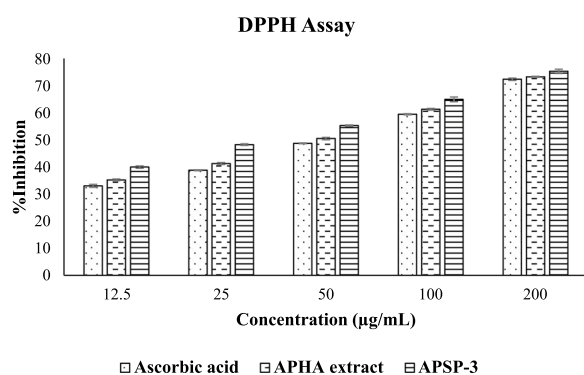
152.010, 131.904, 128.190, 123.837, 116.566, 105.154, 103.628, 99.785, 91.692, 77.167, 76.530, 73.149, 69.641, 60.619, 60.029, 56.460. Mass, m/z : 477.2 ($M+1$). Detailed spectrum obtained from FTIR analysis given in Table 1.

1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The isolated compound (APSP-3) exhibited excellent scavenging activity which is directly proportional to the concentration (Fig. 2). The scavenging activity of ascorbic acid and APHA extract at the highest concentration

Table 1 List of band assignments for FTIR spectrum of APSP-3

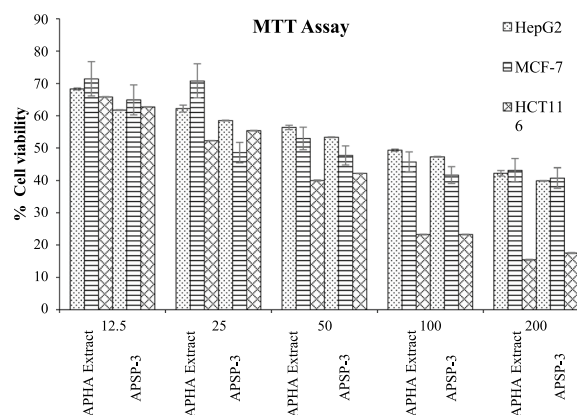
A wave number of the dominant peak (cm ⁻¹)	Functional groups and bond stretching
3780.6	Medium, sharp OH stretching alcohol
3340.82	Strong, broad OH stretching alcohol
2931.9	C–H stretching alkane
1658.84	C=C stretching alkene
1604.83	C=C stretching conjugated alkene
1465.95	C–H bending alkane methylene group
1357.93	OH bending alcohol
1303.92	OH bending phenol
1249.91	C–O stretching aromatic ester
1195.91	Tertiary alcohol or ester
1087.89	Primary-OH Stretching
1041.6	CO–O–CO stretching
833.28	C=C bending
655.82	C=C bending alkene

**Fig. 2** % Inhibition of DPPH radical scavenging activity**Table 2** IC₅₀ values obtained from DPPH assay

Sample	IC ₅₀ value (µg/mL)
Ascorbic acid	75.19 ± 1.5
APHA extract	65.61 ± 1.3
APSP-3	38.70 ± 3.5

Values are represented as mean ± SE (n = 3)

(200 µg/mL) was found 72.31 ± 0.44, 73.22 ± 0.44%, respectively. In comparison, APSP-3 at the same concentration was able to scavenge higher DPPH radicals with a % inhibition of 75.24 ± 0.80. The IC₅₀ values for ascorbic acid, APHA extract, and APSP-3 were 75.19 ± 1.5, 65.61 ± 1.3 and, 38.70 ± 3.5 µg/mL, respectively (Table 2). The lowest IC₅₀ value represents the antioxidant potential of APSP-3 witnessing applicability in drug formulation to combat the disease caused by harmful free radicals.

**Fig. 3** Cytotoxicity assessment of APHA extract and APSP-3

Cytotoxicity assessment using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay

The cytotoxic impacts were measured by % cell viability and its IC₅₀ values were calculated from dose responsive curve. The study revealed a decline in % cell viability in a concentration-dependent manner (Fig. 3, Additional file 2: S1-S4). The APHA extract evince cell viability of 42.25, 43.12 and 15.38% (Table 3) at the highest concentration of 200 µg/mL with IC₅₀ value 122.09, 122.61 and 48.08 µg/mL for HepG2, MCF-7, and HCT116 cell lines, respectively. In contrast, APSP-3 showed 39.89% yielding of IC₅₀ value 96.75 µg/mL for HepG2 followed by 40.75 and 17.50% cell viability with IC₅₀ values of 61.67 and 47.61 µg/mL (Table 4) for MCF-7 and HCT116 cell lines.

Discussion

Thin layer chromatography-direct bioautography (TLC-DB) is often used as a bio-guiding method to scrutinize substances with biological activity that can be further analyzed by spectroscopic methods to obtain information on their structure. Usually, active components found in the analytical scale TLC-DB should be acquired in sufficient amounts and purity for spectroscopic evaluation [32].

Mass analysis of APSP-3 showed major molecular ion *m/z* 477.2 [M + 1]⁺ corresponding to the molecular formula C₂₃H₂₄O₁₁ by using ESI-positive mode (Fig. 4). The data obtained from the analysis revealed the mass fragments of APSP-3, which were interpreted and assigned their respective molecular weight, molecular formula, and chemical structures as presented in Table 5.

A comprehensive CHNS/O analysis revealed that the isolated APSP-3 compound comprises 51.96% carbon, 5.430% hydrogen, and 38.861% oxygen. However, no

Table 3 Cell line specific % cell viability after an exposure of APHA extract and APSP-3

Concentration ($\mu\text{g}/\text{mL}$)	% Cell viability					
	HepG2		MCF-7		HCT 116	
	APHA	APSP-3	APHA	APSP-3	APHA	APSP-3
12.5	68.33 $\pm$ 0.30	61.77 $\pm$ 0.12	71.43 $\pm$ 5.28	64.93 $\pm$ 4.66	65.84 $\pm$ 0.10	62.74 $\pm$ 0.10
25	62.24 $\pm$ 1.06	58.51 $\pm$ 0.13	70.81 $\pm$ 5.23	48.57 $\pm$ 3.17	52.27 $\pm$ 0.06	55.37 $\pm$ 0.10
50	56.40 $\pm$ 0.65	53.34 $\pm$ 0.12	52.95 $\pm$ 3.49	47.69 $\pm$ 2.96	40.00 $\pm$ 0.18	42.21 $\pm$ 0.06
100	49.31 $\pm$ 0.33	42.27 $\pm$ 0.10	45.67 $\pm$ 3.09	41.65 $\pm$ 2.50	23.23 $\pm$ 0.11	23.23 $\pm$ 0.10
200	42.25 $\pm$ 0.84	39.89 $\pm$ 0.10	43.12 $\pm$ 3.61	40.75 $\pm$ 3.17	15.38 $\pm$ 0.18	17.50 $\pm$ 0.14

Values are represented as mean $\pm$ SE ($n=3$)

Table 4 The IC_{50} values of APHA extract and APSP-3 on selected cell lines

Samples	IC_{50} value ($\mu\text{g}/\text{mL}$)		
	HepG2	MCF-7	HCT116
APHA extract	122.09	122.61	48.08
APSP-3	96.75	61.67	47.61

traces of sulfur or nitrogen elements were detected in the compound (Additional file 1: Fig. S1).

The ^1H NMR analysis result observed different proton peaks at upfield and downfield in the NMR spectrum of APSP-3 (Fig. 5, Additional file 2: S2-S4). The number of peaks indicates different types of protons are present in the molecule. The interpretation of spectra covers a total

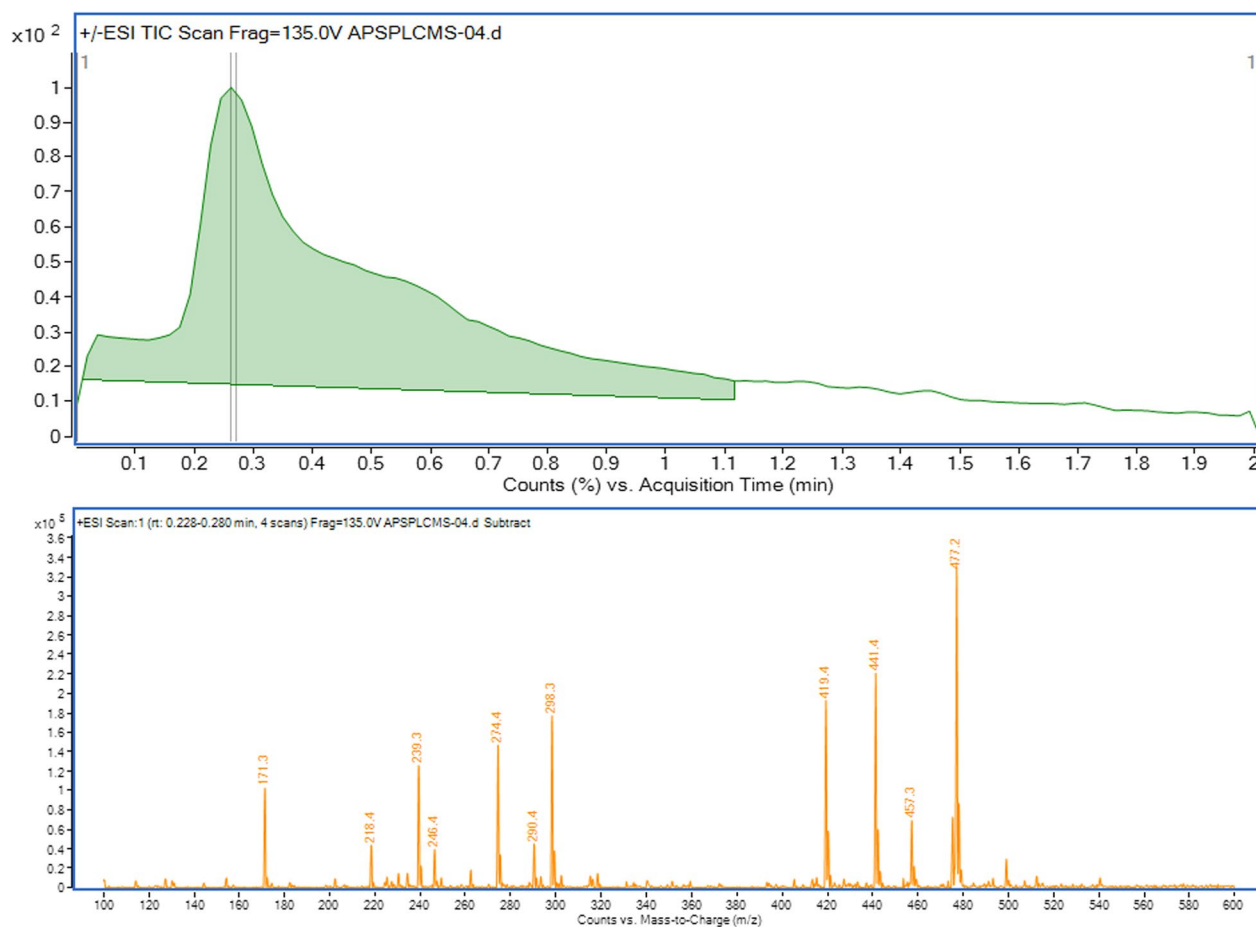
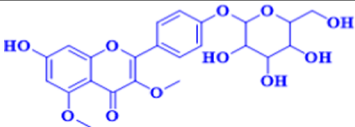
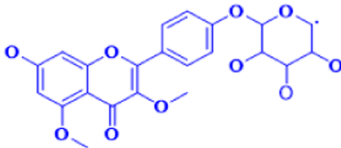
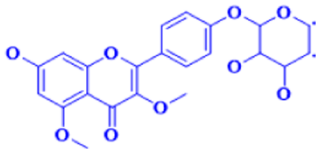
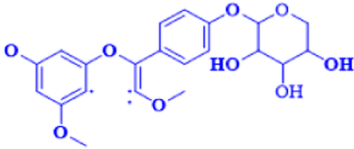
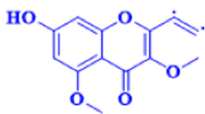
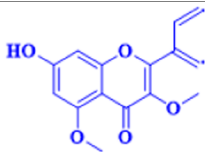
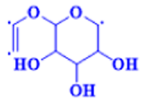
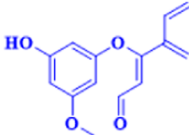
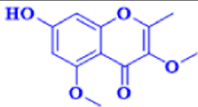
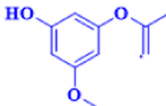
**Fig. 4** LCMS spectrum of APSP-3

Table 5 Mass fragmentation of APSP-3

Plausible Fragments	Molecular Weight (Dalton)	Molecular Formula
	476.43	C ₂₃ H ₂₄ O ₁₁
	441.37	C ₂₂ H ₁₇ O ₁₀ •
	425.37	C ₂₂ H ₁₇ O ₉ •
	417.12	C ₂₁ H ₂₁ O ₉ •
	246.22	C ₁₃ H ₁₀ O ₅ •
	272.26	C ₁₅ H ₁₂ O ₅ •
	173.14	C ₇ H ₉ O ₅ •
	246.26	C ₁₃ H ₁₃ O ₃ •
	236.22	C ₁₂ H ₁₂ O ₅
	179.07	C ₁₀ H ₁₁ O ₃ •

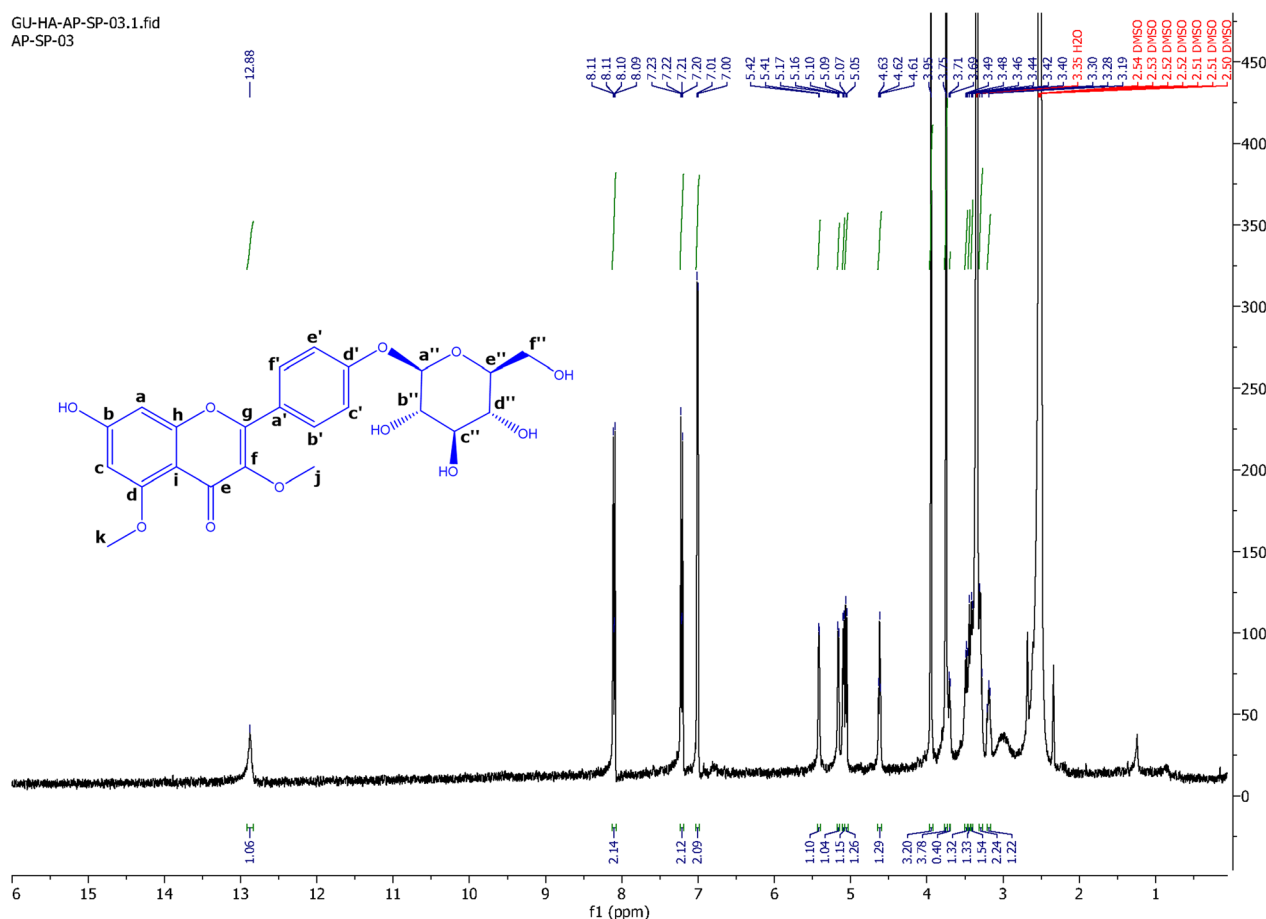


Fig. 5 ^1H NMR spectrum of APSP-3

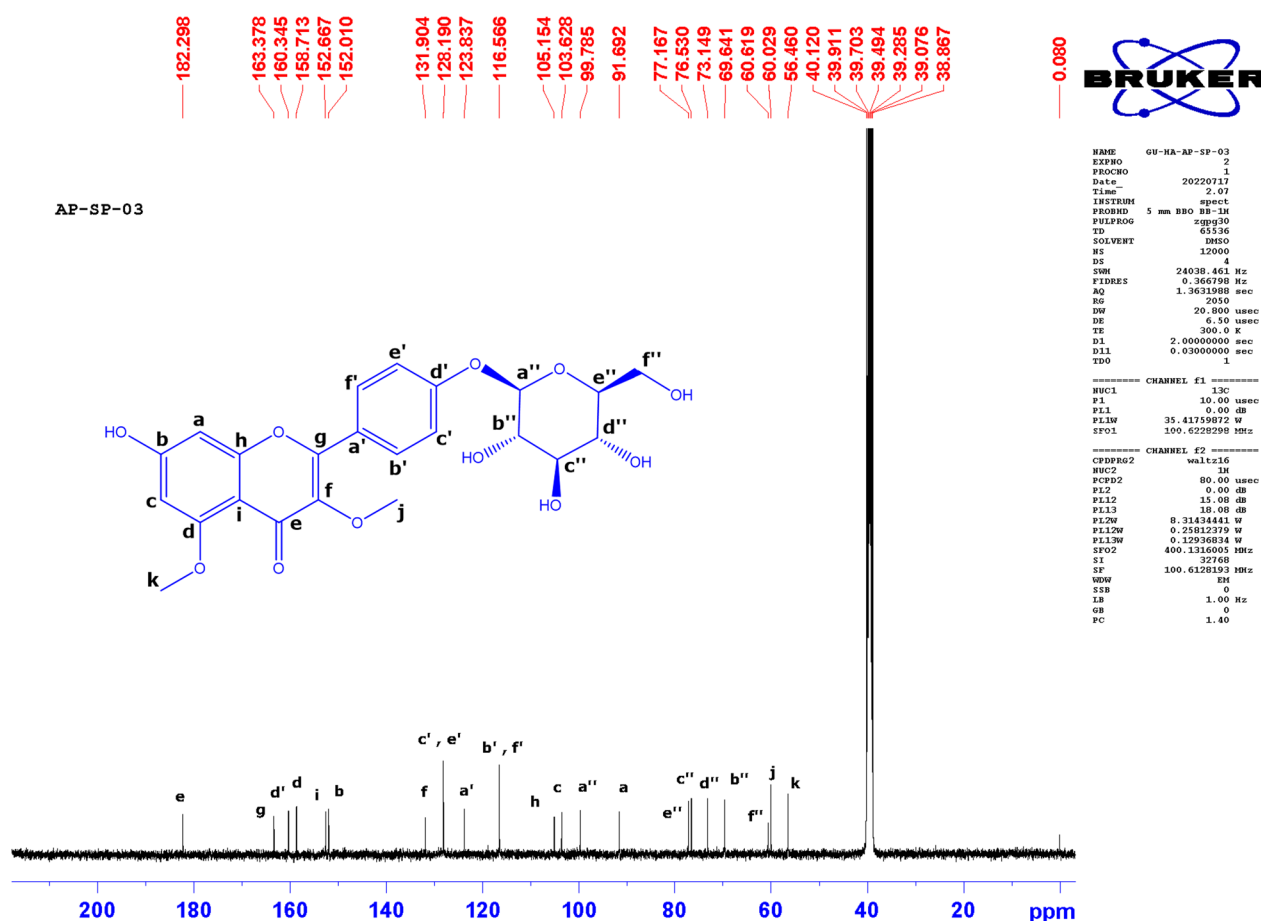
of 17 peaks for protons excluding $\text{DMSO}-d_6$ and moisture traces. The splitting of peaks observed by neighbor proton–proton coupling indicates the surrounding arrangement of protons. The highly deshielded singlet peak at δ 12.88 indicates the presence of an aromatic -OH peak. An aromatic region observed 4 peaks of total six aromatic protons, δ 8.11–8.09 doublet of doublet for two protons, δ 7.23–7.20 doublet of doublet for two protons, δ 7.01 singlet for one proton, and δ 7.00 singlet for one proton. The NMR analysis in MeOD solvent indicates different two singlet peaks near δ 6.7, which appears as a close doublet at δ 7.00 in the $\text{DMSO}-d_6$ spectrum (Additional file 1: Fig. S6). The aliphatic protons of molecules appear at δ 5.42–3.19 with a different neighbor splitting pattern. The D_2O -exchange spectrum indicates the 5-exchangeable protons are present in the molecules, which at δ 12.88, 5.42–5.41, 5.17–5.16, 5.10–5.09 and 4.63–4.61 ppm, respectively, and they are of hydroxy group protons (Additional file 1: Fig. S5).

The ^{13}C NMR observed different peaks at upfield and downfield in the spectrum of ASAP-3 (Fig. 6, Additional file 2: Fig. S7–S10). The interpretation described a total

of 21 peaks excluding $\text{DMSO}-d_6$ at δ 40 ppm. They indicate different types of carbon are present in the molecule. An aromatic range δ 190–90 ppm observed peaks indicate aromatic or group carbons present in the molecule. Similarly, the aliphatic range below δ 80 ppm peaks defines aliphatic carbons. According to the interpretation of peaks, ring-A, ring-B, and ring-C carbons appear at above δ 90 ppm values, while ring-D carbons and methyl carbons appear in the aliphatic region.

The various analyzed 2D NMR analyses include HMQC, HMBC, COSY, DEPT 135 and NOESY for APSP-3. The results constructed plausible chemical structure of isolated compound from the extract of *A. precatorius*.

HMQC is used to assign proton–carbon single bond correlations. The ring-D carbons and protons show the contour for single bond correlations, which are assigned in Fig. 7(I). Similarly, ring-A and ring-C observed contours for correlations of aromatic carbons and protons as shown in Fig. 7(II) (Additional file 1: Fig. S11). HMBC is used to assign proton–carbon multiple bond correlations. The ring-D carbons and protons observed contour,



which indicates the multiple bond correlations as shown in Fig. 7(III, Additional file 1: Fig. S13). Similarly, methyl protons displayed symmetric self-contour pattern at δ 60.029 (j-C) and δ 56.460 (k-C) ppm which confirms their presence in the compound as shown in Fig. 7 (IV, Additional file 1: Fig. S14).

The COSY spectrum indicates correlations through the chemical bond that are two, three, or sometimes four bonds proton with resonances by a mutually coupled nucleus. A clear correlation of δ 8.1 protons (e'-H & c'-H) with δ 7.2 protons (b'-H & f'-H) and splitting doublet of doublet confirm their neighbor position on 1,4-disubstituted aromatic ring-C as shown in Fig. 8(II) (Additional file 1: Fig. S15-S18). Among them, aliphatic C-H protons and hydroxy group protons correlate properly with their splitting pattern which executes the neighbor arrangement in ring-D.

Furthermore, DEPT-135 resonates inverse methylene carbon ($-\text{CH}_2$, f''-C) peak at δ 61.122 ppm, which indicates that only one $-\text{CH}_2$ carbon is present (Fig. 8 III, Additional file 1: Fig. S27). This methylene carbon

(f''-C, δ 61.122) shows contour in HMQC with two separate protons of methylene at near δ 3.45 and δ 3.69 ppm, respectively (Fig. 7 I, Additional file 1: Fig. S12). COSY correlations of the above two methylene protons indicate they are coupled by adjacent hydroxy proton which is triplet at δ 4.62 ppm (Fig. 8 I, Additional file 1: Fig. S16). The NOESY response is low intensive during an experiment, the position of methyl proton (k-C) is confirmed based on only one single correlation with a ring-A aromatic proton (Additional file 1: Fig. S19-S26).

The IR spectrum of the compound has been analyzed to elucidate the presence of various bonds and groups as shown in Fig. 9. In the spectrum, the presence of broad bands at 3780.60 cm^{-1} and 3340.82 cm^{-1} represents medium, sharp OH stretching alcohol and strong, broad OH stretching alcohol, respectively. The C-H stretching alkane was observed at 2931.90 cm^{-1} , while peak at 1658.84 cm^{-1} showed C=C stretching alkene.

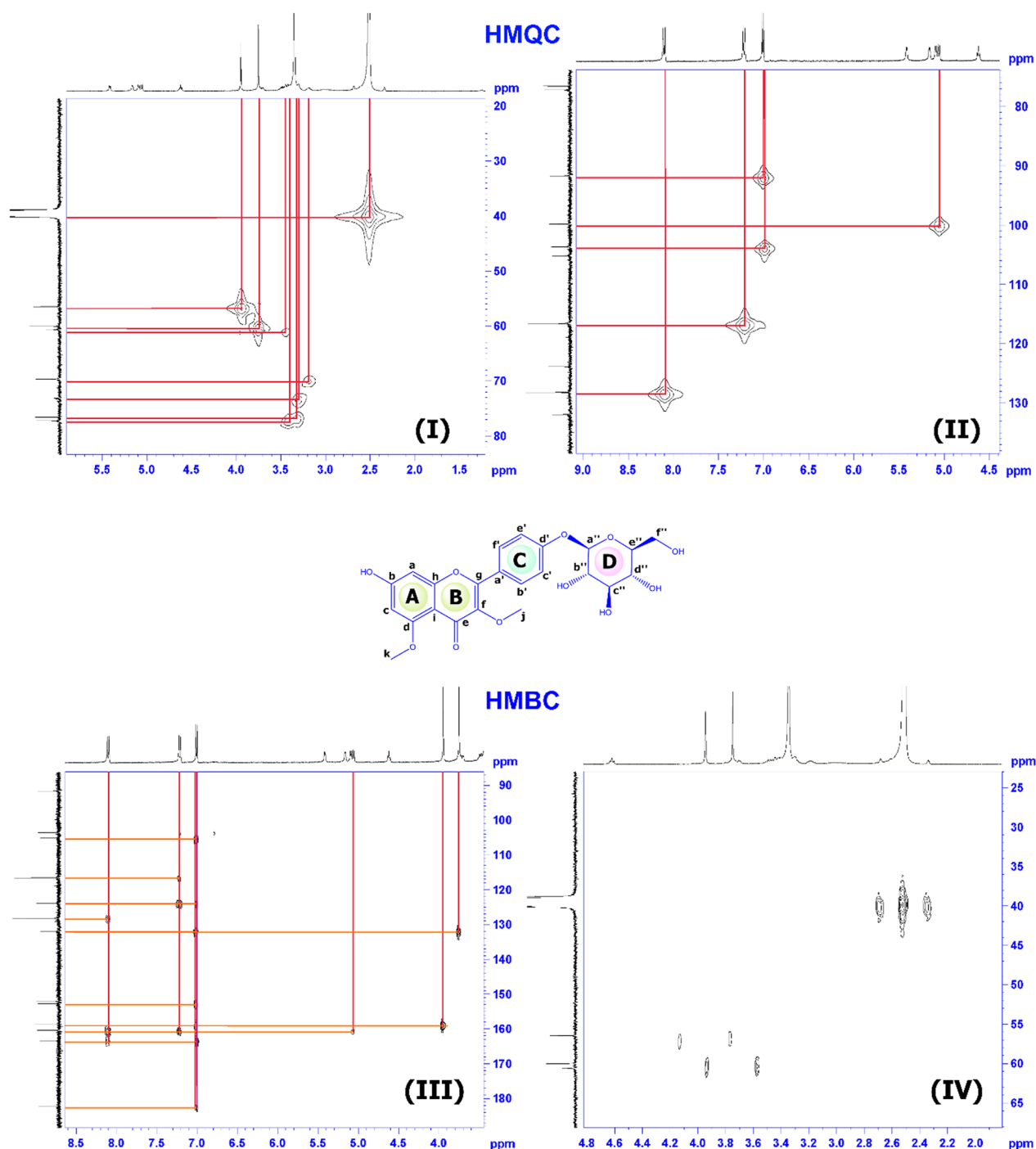


Fig. 7 HMQC (I, II) and HMBC (III, IV) spectrum of APSP-3

Based on the analysis of the data, it has been concluded that the proposed structure is composed of four rings, as portrayed in Fig. 10.

DPPH assay is a rudimentary parameter to check the antiradical activity of natural compounds [33] and

cumulative findings [34] proposed that the antioxidant potential of natural compounds stipulates the presence of phenolic and flavonoid components. The higher percentage of scavenging activity and lower IC_{50} values usually imply greater antioxidant activity. The antioxidant efficacy of flavonoids attributed to their functional

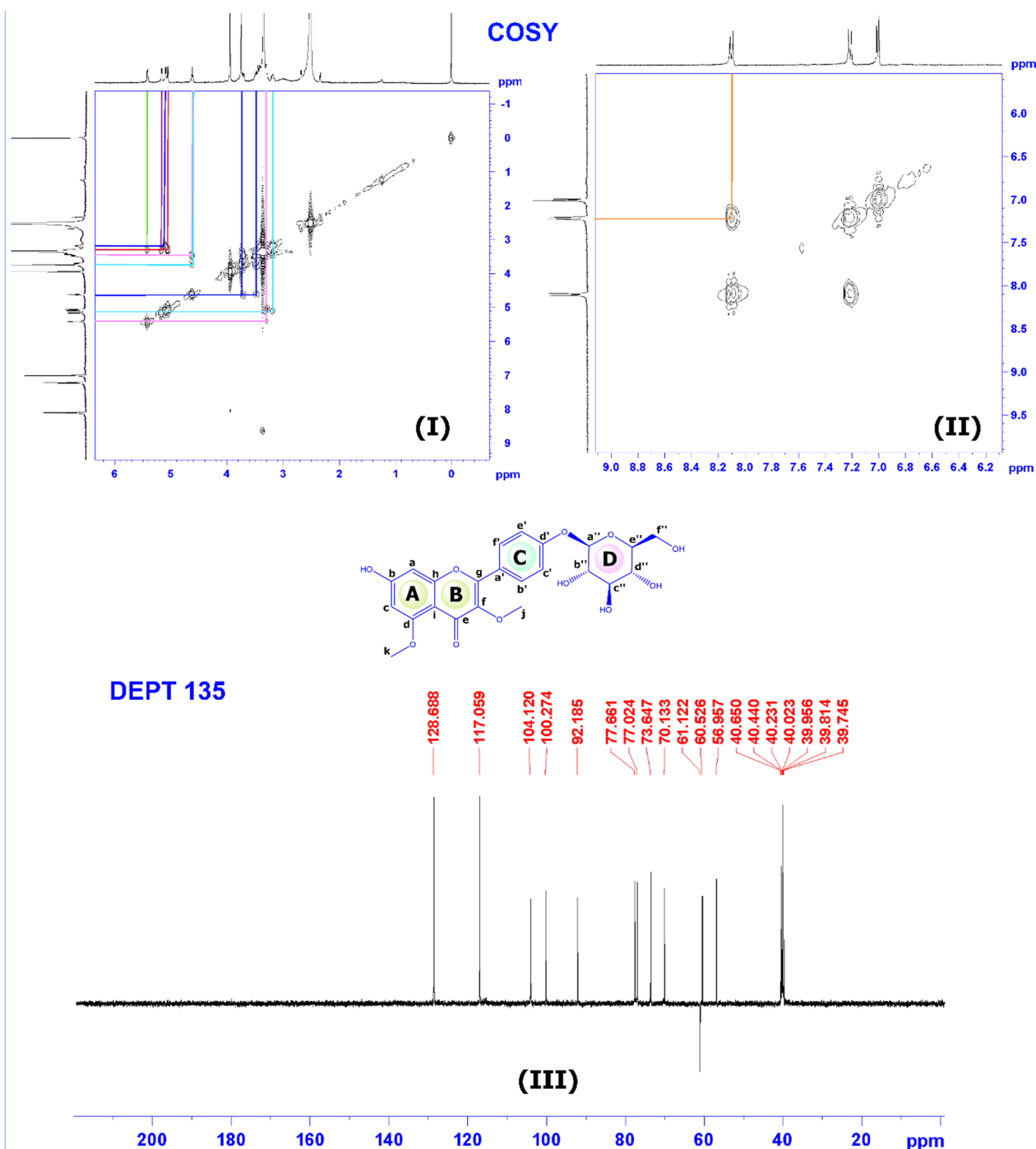


Fig. 8 COSY (I, II) and DEPT-135 (III) spectrum of APSP-3

hydroxyl groups plays pivotal role in counteracting free radicals and/or by chelating metal ions [35]. In this study, APSP-3 exhibited higher antioxidant potency than APHA extract and standard ascorbic acid too. Higher scavenging activity of isolated flavonoid as compared

to standard compounds was also observed by other researchers [36, 37].

In order to evaluate the cytotoxicity and dose response effect for PHA extract and particularly for isolated compound (APSP-3), HepG2, MCF-7, and HCT116 cell lines were employed. The benchmark for cytotoxicity

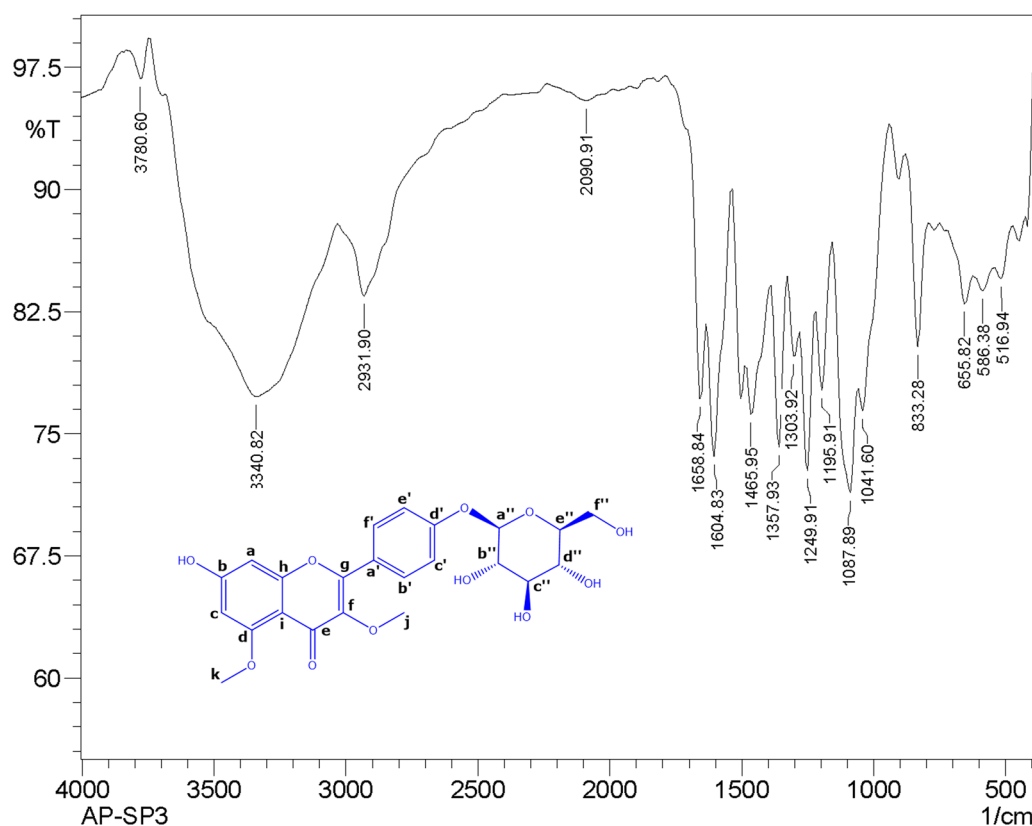


Fig. 9 FTIR spectrum of APSP-3

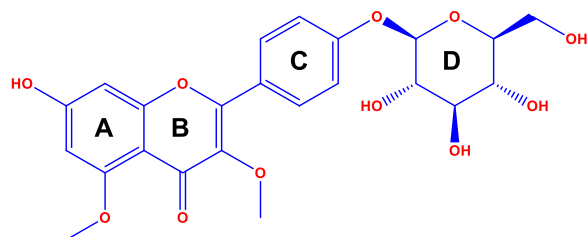


Fig. 10 Structure of isolated compound APSP-3

for the crude extracts determined by the NCI is an IC_{50} value $< 30 \mu\text{g/mL}$. However, in the present investigation, APHA extract as well as APSP-3 compound both divulge IC_{50} value $> 30 \mu\text{g/mL}$ at the studied concentration on selected cell lines. Our finding is supported by the recent study carried out on the cytotoxic effect of *A. precatorius* leaves on different cell lines [38].

Conclusion

The significance of plants lies in their bioactive constituents, which possess properties capable of influencing biological systems, including human health. Consequently, the need for activity-guided phytopharmacological

evaluation becomes evident. In the present study, a new biologically active flavonoid was isolated from the APHA extract based on a HPTLC-DPPH bioautography-guided strategy. The structure of flavonoid was elucidated by various high-throughput spectroscopic techniques and named 7-hydroxy-3,5-dimethoxy-2-(4-((3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl) oxy) phenyl)-4H-chromen-4-one. This flavonoid unveils significant free radical scavenging activity ($IC_{50} = 38.70 \pm 3.5 \mu\text{g/mL}$). The cytotoxicity assessment is not critical step only in evaluating the safety profile of the compound, but also offers valuable insights into the dose–response relationship, aiding in determining the concentration at which the substance initiates toxic effects. Thus, the present study portrays integrated process for the identification of bioactive compounds from natural sources, providing a detailed understanding of their structures and potential therapeutic effects. In this context, additional exploration of diverse cell lines is requisite to stamp out cytotoxicity of the compound. Further research is required to investigate additional bioactivities encompassing in silico and in vivo

studies obliged to provide a robust and secure portrayal for clinical applications.

Abbreviations

APIs	Active pharmaceutical ingredients
<i>A. precatorius</i>	<i>Abrus precatorius</i>
HPTLC	High-performance thin layer chromatography
DPPH	1,1-Diphenyl-2-picrylhydrazyl
TLC-DB	Thin layer chromatography-direct bioautography
APHA	<i>Abrus precatorius</i> hydroalcoholic extract
NMR	Nuclear magnetic resonance
FTIR	Fourier transform infrared spectroscopy
HPLC–MS/MS	Liquid chromatography-tandem mass spectrometry
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HMQC	Heteronuclear multiple quantum correlation spectroscopy
NOESY	Nuclear Overhauser effect spectroscopy
COSY	Correlation spectroscopy
DEPT-135	Distortionless enhancement by polarization transfer
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide
NADH	Nicotinamide adenine dinucleotide
KBr	Potassium bromide
MeOD	Deuterated methanol
DMSO	Dimethyl sulfoxide
TMS	Tetramethylsilane
NCCS	National Centre for Cell Sciences
DMEM	Dulbecco's modified Eagle's medium
FBS	Fetal bovine serum
PBS	Phosphate-buffered saline
CO ₂	Carbon dioxide
IC ₅₀	Half maximal inhibitory concentration
DW	Distilled water
h	Hours
mM	Millimolar
mL	Milliliter
mg	Milligram
µg	Microgram
min	Minutes
cm	Centimeter
mm	Millimeter
nm	Nanometer
°C	Celsius
S/N	Serial number
v/v	Volume/volume
MHz	Megahertz
ESI	Electrospray ionization
TEM	Temperature
DP	Declustering potential
CE	Collision energy
CES	Collision energy spread
<i>m/z</i>	Mass/charge
SE	Standard error

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-023-00571-4>.

Additional file 1. Supplementary figures s1–s29.

Additional file 2. Supplementary figures s1–s5.

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Author contributions

HA performed and interpreted the data regarding the HPTLC screening, bioautography spectrophotometric antioxidant assay, as well isolation and purification and have a major contribution to manuscript writing. RD immensely helped in structural elucidation of isolated compound and also supported in manuscript writing. PT and MT contributed in toxicity analysis. JM was contributed in supervising spectroscopic analysis. GS was associated with supervising, advising, and structuring the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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