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Comprehensive investigation of antibacterial, cytotoxic, and antioxidant activities of *Ipomoea staphylina* flower extract with isolation and characterization of Beta-sitosterol

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ABSTRACT

This study aims to bridge the gap by scientifically investigating the ethnopharmacological evidence surrounding the *I. staphylina* plant. In this research we evaluated the antibacterial and cytotoxic abilities of *I. staphylina* flower extracts. The extract with the highest antioxidant activity underwent cytotoxicity testing against HCT-116 cells. Further analysis confirmed the presence of Beta-sitosterol through HPTLC, followed by its isolation via column chromatography. Antibacterial tests showed significant activity against various gram-positive and gram-negative bacteria, with notable resistance against *S. aureus, P. mirabilis*, and *P. aeruginosa*. The IPFPE extract exhibited the highest inhibition against *S. aureus* (16 mm) and *P. mirabilis* (15 mm), while ethanolic extract showed potent activity against *S. aureus* and *P. aeruginosa* (16 mm, 15 mm). The anticancer study, using ethanolic and pet-ether extracts on HCT-116 colon cancer cells and assessed via the MTT assay, revealed significant reductions in cell viability due to apoptosis induced by secondary metabolites (Beta-sitosterol) and antioxidants. A significant reduction in cell viability was observed, with IC_{50} values of 51.44 μM (IPFE) and 52.74 μM (IPFPE), a 50 % growth inhibition was observed, highlighting vigorous pro-apoptotic activity. These results underscore the potential of *I. staphylina* flower extracts as promising antibacterial and chemotherapeutic agents, warranting further research to validate their efficacy.

1. Introduction

Herbal plants have served as valuable resources for centuries in the management and treatment of a wide array of diseases [1,2]. The utilization of medicinal plants, as well as the isolation and study of their bioactive compounds, has profoundly influenced the realms of both traditional and alternative medicine throughout millennia [3,4]. These botanical sources have consistently offered a rich reservoir of natural substances with therapeutic potential, making them subjects of great significance in the fields of pharmacology and healthcare [3,4]. Herbal medicines have emerged as fundamental components of diverse alternative therapeutic modalities that have witnessed a surge in popularity

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in recent years [5]. These botanical remedies exert a significant influence on the landscape of pharmaceutical research and design [5–7]. The growing adoption of herbal-based treatments can be attributed to their notable efficacy, cost-effectiveness, reduced toxicity, and environmentally sustainable attributes [8]. Intriguingly, a substantial proportion of pharmaceutical agents employed in the treatment of conditions ranging from cancer to infectious diseases, approximately 60 % to 75 %, find their origins in natural sources [8–11]. These herbal remedies, recognized for their capacity to interact with various biological proteins, hold considerable value in the realm of drug design, actively contributing to the development of innovative pharmaceutical compounds [11,12].

Ipomoea staphylina Rome & Schult, a prominent climbing plant, has been a vital constituent of traditional medicine with a rich history of treating diverse health issues. Its traditional applications encompass the management of respiratory conditions, helminth infections, bronchitis, and gastrointestinal disorders [13,14]. This plant species boasts a wide spectrum of pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, anti-diabetic, anti-ulcer, and anti-mutagenic effects [14]. Recent research has shown the plant's impressive capability in synthesizing nanoparticles, especially in the biosynthesis of silver and CuO nanoparticles utilizing its extract as a natural doping agent. Moreover, quercetin has been effectively isolated from the plant extract. The synthesized nanoparticles show considerable larvicidal effects on mosquito larvae, underscoring their potential uses in vector management and biomedical studies [15–17]. Notably, residents of Gingee Hills have harnessed the therapeutic potential of leaf latex to address concerns like foot cracks [15–18]. Furthermore, the roots of *I. staphylina* have been employed as an antidote for snake bites by the Irula and Palliyar tribes [19]. In the vicinity of Karandamalai, villagers have traditionally turned to a leaf decoction to alleviate stomach ailments [20]. Simultaneously, the Chenchus tribes have leveraged leaf extracts as a remedy for piles [21]. These longstanding traditional applications underscore the plant's multifaceted therapeutic potential across diverse cultural contexts. Despite the reported pharmacological activities of *I. staphylina* and its extensive traditional uses, its flower remains an uncharted territory in scientific research. This is notably due to the rarity of *I. staphylina* flowers, which are only available during the period from end of the December to February [13,14]. Recognizing the plant's well-documented medicinal properties, both within traditional and non-traditional systems, our research aims to explore the biological activities of *I. staphylina* flowers, grounded in the existing traditional evidence.

Our research involves the successful extraction of *I. staphylina* flowers, followed by a thorough traditional pharmacological evaluation to unveil the flower's potential [22,23]. Given the traditional use of *staphylina* for inflammation and piles, we aim to delve into the plant's biological attributes in related areas, such as antibacterial activity, antioxidant properties, and its potential as an anticancer agent against colon cancer cells, specifically HCT-116 [13–21]. The extract's quality was meticulously assessed by quantifying its phytochemical and biologically active components, with a focus on those presenting the highest concentration [22–25]. Subsequently, the extract displaying the most substantial antioxidant activity was subjected to cytotoxicity testing against HCT-116 cells. Following a thorough preliminary analysis, the presence of Beta-sitosterol was strongly confirmed using HPTLC. This compound was then isolated through column chromatography and underwent comprehensive characterization employing advanced analytical techniques such as NMR, GCMS, and FTIR [26,27]. The chromatographic profiles of the identified compounds closely paralleled published literature, thus solidifying their identification.

2. Results and discussion

2.1. Pharmacognostical analysis

The pharmacognostic evaluation of plants is an inestimable instrument in the identification of drug to prevent adulteration [22,23,28–32]. The analysis of *I. staphylina* flower powder indicated significant phytochemical characteristics (Supplementary Table S2). The moisture content, indicated by the loss on drying, was 10.0 %. The total ash value, representing the inorganic residue after combustion, was 18.8 %. The water-soluble ash content was 82.45 %, while the acid-insoluble ash, indicating siliceous matter, was 17.5 %. These findings offer valuable insights into the extract's composition and quality, supporting its potential use in further pharmacological studies.

I. staphylina flower powder was subjected to treatment with various solvents and subsequently observed (Supplementary Table S1, Supplementary Fig. S2) under UV light at different wavelengths (Supplementary Fig. S2). Fluorescence analysis was performed to ascertain the purity of the herbal drug. In essence, pharmaconostical analyses serve as invaluable tools in the realm of pharmacognosy, enabling the comprehensive evaluation of the purity, authenticity, and overall quality of herbal drugs and natural products. These determinations play a pivotal role in the processes of standardization, safety assessment, and the formulation of efficacious herbal medicines [28,29]. This multidimensional approach aids in ensuring the effectiveness, safety, and reliability of herbal products within the pharmaceutical and healthcare industries.

Qualitative phytochemical analysis helps identify the presence of various bioactive compounds, such as alkaloids, flavonoids, tannins, terpenoids, and glycosides, present in plant extracts. Knowing the chemical constituents of plants aids in understanding their potential medicinal properties and pharmacological activities [22,23]. Qualitative phytochemical results shows (Supplementary Table S3) that all the three extracts have the phenols, flavonoids, terpenoids. Sterols very less amount present in all three extracts. Saponins and carbohydrates only present in aquas extract. Also, alkaloids only present in aquas and ethanolic extract.

Subsequent to the qualitative analysis, a quantitative assessment was carried out to precisely determine the concentration of secondary metabolites within the samples [30]. Quantitative phytochemical estimation was conducted in accordance with established protocols and utilizing a UV photo spectrometer [30,31]. The results (Table 1) elucidated that IPFPE exhibited a notably elevated content of flavonoids. In contrast, IPFE displayed a substantially higher concentration of phenolic compounds compared to the other extracts. Notably, IPFAE was the sole sample found to contain carbohydrates, demonstrating the diverse phytochemical profiles of the different extracts. This quantitative analysis provides a quantitative understanding of the specific bioactive compounds present in these extracts, which is fundamental in elucidating their potential health benefits and applications.

2.2. Antibacterial activity

The plant extracts subjected to examination of antibacterial activity against selected bacteria. It displayed potent antibacterial properties against a spectrum of bacteria, encompassing *S. aureus, P. mirabilis, P. aeruginosa, E. coli, and* K. *pneumonia*.

The IPFPE extract exhibits remarkable antibacterial activity against (Fig. 1a), (Table 2) both gram-positive and gram-negative strains. Notably, at a concentration of 100µg/ml, it demonstrates a substantial zone of inhibition measuring 16 mm against *S. aureus*. Additionally, Pet-ether extract exhibits a 15 mm zone of inhibition against *P. mirabilis* and

Table 1
Quantitative phytochemical analysis *I. staphylina* flower extracts.

S. No	Sample code	Total Phenolics (mg/g)	Total Flavonoids (mg/g)	Total Tannins (mg/g)	Total Carbohydrates (mg/g)
1	IPFPE	35.35	844.65	193.525	-
2	IPFE	163.4722	321.7745	-	-
3	IPFAE	32.73889	206.796	187.275	360.335

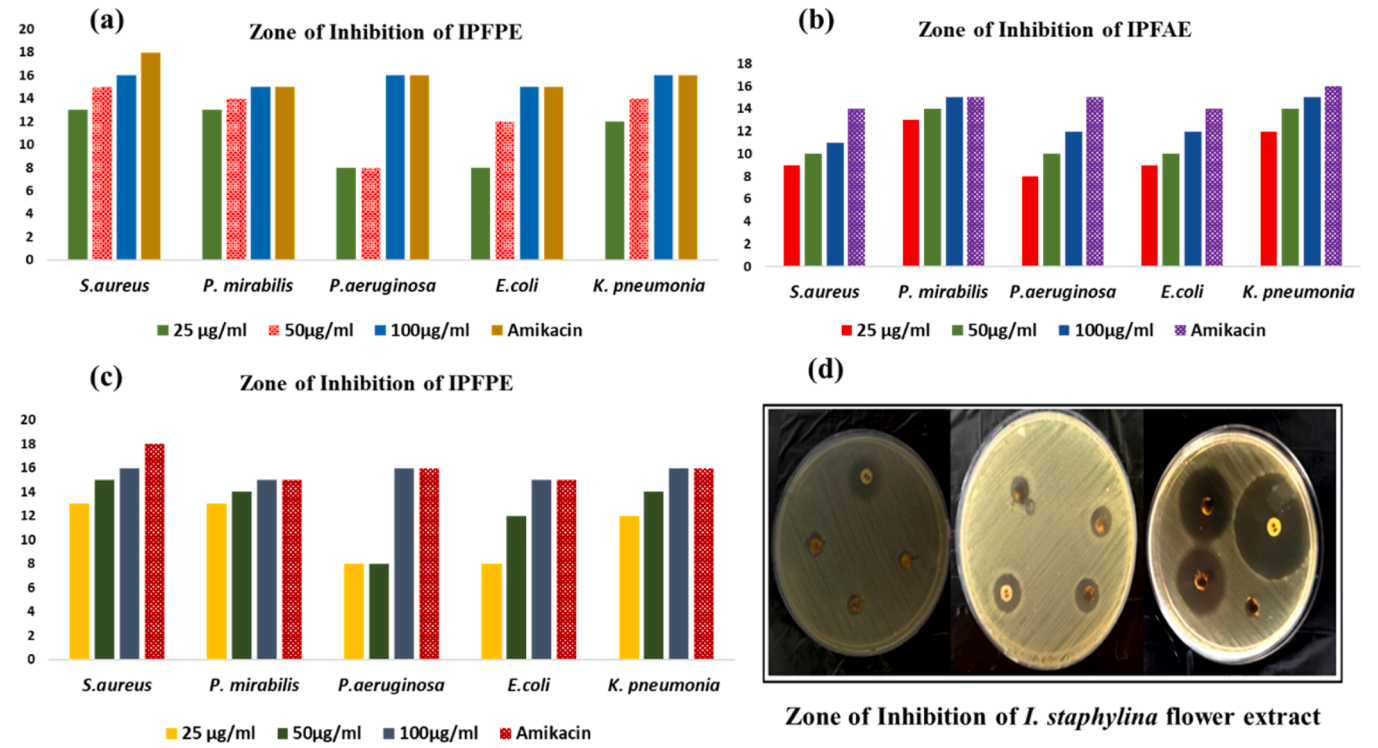

Fig. 1. (**a**) Zone of Inhibition of IPFPE sample, (**b**) Zone of Inhibition of IPFAE sample, (c) Zone of Inhibition of IPFPE sample and (d) Zone of Inhibition of *I. staphylina* flower extracts.

Table 2
Zone of inhibition concentration (mm) for various concentration of IPFPE.

S.No	Bacteria	IPFPE (various concentration)			Amikacin
		25 µg/ml	50µg/ml	100µg/ml	
1	*S. aureus*	13	15	16	18
2	*P. mirabilis*	13	14	15	15
3	*P. aeruginosa*	8	8	16	16
4	*E. coli*	8	12	15	15
5	*K. pneumonia*	12	14	16	16

a 14 mm zone of inhibition against *P. aeruginosa*. Furthermore, it displays excellent inhibitory effects with a 15 mm zone of inhibition against *E. coli* and a 16 mm zone of inhibition against K. *pneumonia* at the same concentration of 100µg/ml. These findings underscore the potent antibacterial properties of the IPFAE extract.

Specifically, the zone of inhibition exhibited by IPFE against *S. aureus* measured 16 mm at 100µg/mL and 15 mm at 50µg/mL. In the case of *P. mirabilis*, it manifested zones of 11 mm at 100µg/mL and 10 mm at 50µg/mL. Pertaining to *P. aeruginosa*, IPFE demonstrated zones of 15 mm at 100µg/mL and 14 mm at 50µg/mL. Similarly, in the context of *E. coli*, it showcased zones of 10 mm at both 100µg/mL and 50µg/mL. In the case of K. *pneumonia*, IPFE presented zones of 10 mm at 100µg/mL and 9 mm at 50µg/mL. These findings (Table 3, Fig. 1c) underscore the extract's robust antibacterial efficacy against a spectrum of bacteria, both gram-positive and gram-negative, effectively rivaling the reference antibiotic, amikacin.

Notably, IPFAE exhibited a remarkable degree of antibacterial efficacy (Table 4, Fig. 1b). Specifically, when tested against *S. aureus*, it generated a zone of inhibition measuring 11 mm at 100 µg/mL and an even more substantial 15 mm at 50µg/mL, indicating a significant inhibitory effect on bacterial growth. In the case of *P. mirabilis*, the extract displayed zones of inhibition measuring 15 mm at 100 µg/ml and 14 mm at 50 µg/ml, reinforcing its potent antibacterial potential. Against *P. aeruginosa*, IPFAE demonstrated considerable antibacterial activity, as evidenced by zones of inhibition measuring 12 mm at 100 µg/mL and 110 mm at 50µg/ml. Similarly, when evaluated against *E. coli* and K. *pneumonia*, IPFAE revealed zones of inhibition measuring 12 mm at 100 µg/mL and 10 mm at 50 µg/mL, and 15 mm at 100µg/mL and 14 mm at 50 µg/mL, respectively. These findings underscore the extract's substantial and versatile antibacterial properties, signifying its potential as a valuable agent in the battle against bacterial infections.

2.3. DPPH assay

The results unequivocally demonstrate the remarkable antioxidant activity of the plant extract (Fig. 2). In this antioxidant assay, Rutin was employed as the standard. Notably, both the pet-ether and ethanolic extracts displayed superior antioxidant activity when compared to the

Table 3
Zone of inhibition concentration (mm) for various concentration of IPFE.

S. No.	Bacteria	IPFE (various concentration)			Amikacin
		25 µg/mL	50µg/mL	100µg/mL	
1	*S. aureus*	14	15	16	18
2	*P. mirabilis*	9	10	11	14
3	*P. aeruginosa*	12	14	15	18
4	*E. coli*	8	10	10	12
5	K. *pneumonia*	8	9	10	14

Table 4
Zone of inhibition concentration (mm) for various concentration of IPFAE.

S. No.	Bacteria	IPFAE (various concentration)			Amikacin
		25 µg/mL	50µg/mL	100µg/mL	
1	*S. aureus*	9	10	11	14
2	*P. mirabilis*	13	14	15	15
3	*P. aeruginosa*	8	10	12	15
4	*E. coli*	9	10	12	14
5	*K. pneumonia*	12	14	15	16

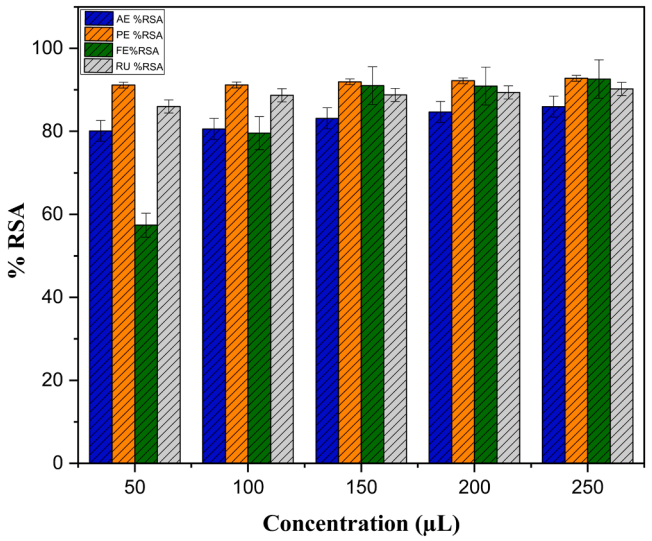

Fig. 2. DPPH assay of *I. staphylina* flower extracts.

standard. IPFE exhibited antioxidant activity with values of 91.0 % RSA at 150µg/mL, 90.91 % RSA at 200µg/mL, and 92.60 % RSA at 250µg/mL. Meanwhile, IPFPE displayed antioxidant activity with values of 91.93 % RSA at 150µg/mL, 92.19 % RSA at 200µg/mL, and 92.79 % RSA at 250µg/mL, whereas the standard showed values of 88.77 % at 150µg/mL, 89.36 % at 200µg/mL, and 90.20 % RSA at 250µg/ml. These findings underscore the superior antioxidant potential of the plant extracts, with IPFE and IPFPE exhibiting exceptional activity.

2.4. Cytotoxic assay

The provided Figs. 3, 4 distinctly illustrates a notable reduction in cell growth subsequent to the treatment with the plant extract samples. Evidently, this reduction signifies the remarkable inhibitory effect of IPFE and IPFPE samples on the proliferation of HCT-116 cells. The data presented in the figure serves as a visual testament to the substantial impact of these plant extracts on impeding the growth of HCT-116 cells

This observation is of great significance within the scope of our research, highlighting the potential of IPFE and IPFPE as potent agents for regulating cell growth, particularly in the context of HCT-116 cells.

The provided Tables 5–7 and Figs. 3, 4 offers a comprehensive overview of the absorption values and cell viability obtained through the MTT assay, comparing the samples to the standard. These values provide crucial insights into the viability of HCT-116 cells, ultimately yielding the IC_{50} values for each sample. Notably, the standard presents an IC_{50} value of 29.25 µM, while IPFE and IPFPE exhibit IC_{50} values of 51.44 µM and 52.74 µM, respectively. These IC_{50} values elucidate the impressive efficacy of both IPFE and IPFPE in effectively suppressing the proliferation of HCT-116 cells, underscoring their potential as formidable candidates for anti-cancer therapeutics. This data highlights the promising anti-cancer properties of the studied plant extracts, potentially contributing to the development of novel and effective anti-cancer agents.

2.5. Isolation

Following the initial qualitative analysis, the plant extracts underwent a comprehensive GCMS analysis aimed at elucidating the precise composition of phytochemicals contained within the plant extract. This analytical approach was undertaken to provide a detailed and scientific insight into the specific bioactive compounds present in the extracts, enhancing our understanding of their chemical constituents and potential applications. Beta-sitosterol was initially predicted based on the GC–MS library match and was subsequently confirmed and isolated using column chromatography.

2.5.1. GCMS results

Subsequent to the GCMS analysis (Supplementary Fig. S3) the results were subjected to prediction via the NIST library (Table 8), revealing a concordance with the library search results and yielding predictions of numerous bioactive compounds. Notably, β-sitosterol was identified at an approximate retention time (RT) value of 29.84, a finding that was

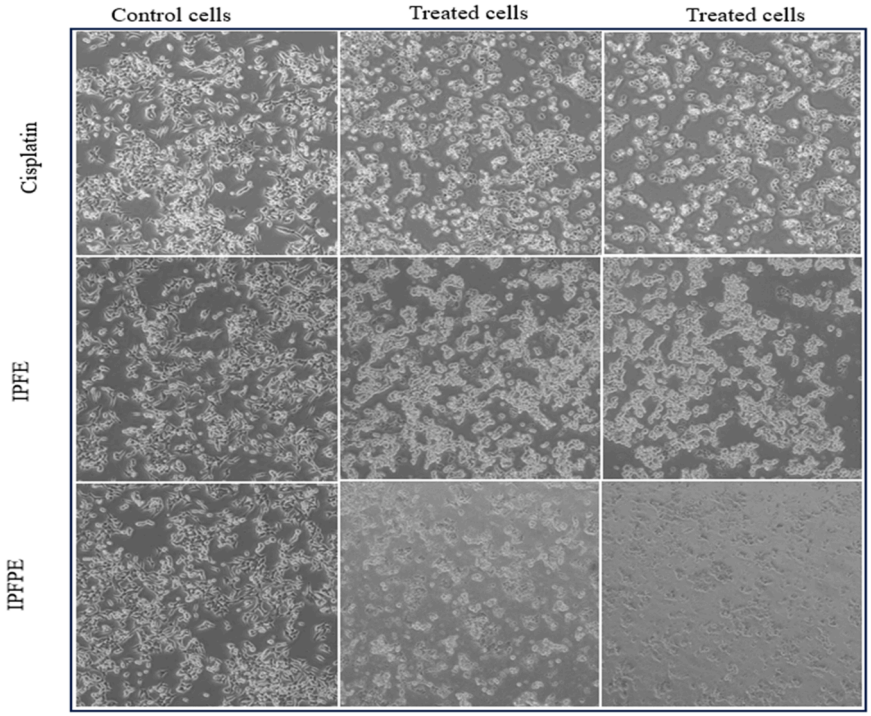

Fig. 3. Images of treated cells and control HCT-116 cancer cells.

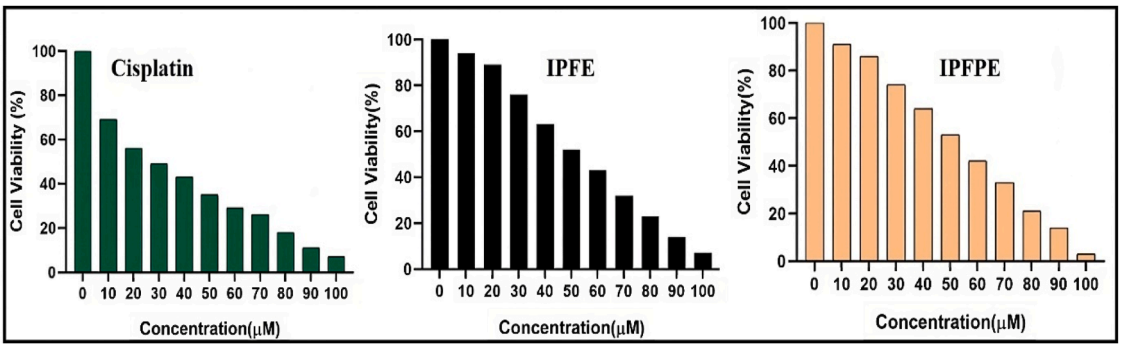

Fig. 4. MTT assay of *I. staphylina* flower extract.

Table 5
MTT assay of standard.

Functions	HCT-116 CELLS							Standard: Cisplatin			
Concentration	0	10	20	30	40	50	60	70	80	90	100
Absorption	1.164	0.799	0.657	0.569	0.499	0.403	0.336	0.3	0.207	0.123	0.080
Cell Viability	100	69	56	49	43	35	29	26	18	11	7
IC_{50} = 29.25 ±0.14 μg/mL											

Table 6
MTT assay of IPFE.

Functions	HCT-116 CELLS							Standard: IPFE			
Concentration	0	10	20	30	40	50	60	70	80	90	100
Absorption	0.997	0.942	0.890	0.753	0.629	0.514	0.430	0.320	0.229	0.139	0.067
Cell Viability	100	94	89	76	63	52	43	32	23	14	7
IC_{50} = 51.44± 0.09 μM											

Table 7
MTT assay of IPFPE.

Functions	HCT-116 CELLS							Standard: IPFPE			
Concentration	0	10	20	30	40	50	60	70	80	90	100
Absorption	0.994	0.903	0.856	0.734	0.635	0.522	0.415	0.332	0.204	0.143	0.033
Cell Viability	100	91	86	74	64	53	42	33	21	14	3
IC_{50} = 52.74± 0.09 μM											

further substantiated through HPTLC analysis. The isolation of β-sitosterol was achieved employing column chromatography. The purity of the isolated compound was subsequently verified through advanced instrumentation techniques, including NMR, FTIR, and GCMS. These comprehensive analyses serve to establish the precise identity of β-sitosterol, reinforcing its significance within the scope of this research.

2.5.2. *Confirmation of Beta-sitosterol*

The HPTLC analysis (Supplementary Fig. S4), (Supplementary Table S4) indicates a matching Rf value (0.6) for the standard and petroleum ether extract, providing clear confirmation of the presence of beta-sitosterol in the sample [32]. Once the presence of beta-sitosterol was established, it was meticulously separated through the application of column chromatography. Subsequently, the isolated compound was further elucidated using advanced instrumentation techniques. Proton and ^{13}C NMR results depicted in the Fig. 5a, b.

The ^{1}H NMR spectrum of **Beta-sitosterol (**400 MHz, DMSO-d_6): The Aliphatic OH proton shows at the range of 5.287 ppm (s, H), C=C—H sows at 3.5 ppm (m, H). The other Aliphatic protons appeared at the range of 2 - 0.8 ppm which is aliphatic region [43–45]. The ^{13}C NMR spectrum of **Beta-sitosterol** (100 MHz, DMSO-d_6, δ) δ carbon–carbon appeared (ethylene) at 140.77, another ethylene peak appeared at 121.72 ppm OH—C appeared 77.33 ppm, rest of the aliphatic carbons appeared at the region of 77.02–11.86 ppm. The ^{13}C spectra confirms the presence of the beta-sitosterol [30–32]. In the FTIR analysis it shows (Fig. 6b) that the OH stretch appeared at the 3437.68 cm^{-1} and alkene carbons C=C stretch shows at the 2930.84, 2863.91 cm^{-1} all the Aliphatic carbons shows at the 1646.70 to 495.20 cm^{-1} which is similar to the available data [33–35]. This FTIR helps to confirm the functional group of Beta-sitosterol. Furthermore, GCMS spectroscopy was employed to precisely determine the molecular weight of this isolated compound. The spectroscopic analysis (Fig. 6a) yielded a molecular ion peak $[M+H]^+$ at approximately 414.717, a value consistent with the molecular weight of Beta-sitosterol. It's noteworthy that these results align seamlessly with the findings reported in prior scholarly research [33–35].

Before commencing any research endeavor, the imperative step of standardizing herbal drugs is addressed [22,23]. In the specific context of *I. staphylina* flower powder, a range of pharmacognostic evaluations were meticulously executed to uphold its quality and genuineness. These encompassed indispensable criteria, encompassing loss on drying, ash value, water-soluble ash, and acid-insoluble ash, with detailed outcomes provided in Supplementary file: Table 1. Such stringent examinations serve the dual purpose of safeguarding against potential adulterants and preserving the authenticity of the herbal drug [28,29]. Additionally, fluorescence analysis (Supplementary Table S2) emerged as a pivotal

Table 8
GCMS predicted compounds from NIST library.

S. No.	Compound Name	Rt Value	Molecular Weight	Molecular Formula
1	Cyclopentanol, 1-Methyl	2.9	100	$C_6H_{12}O$
2	3,4-Hexanediol, 2,5-Dimethyl-	4.8	146	$C_8H_{18}O_2$
3	Ethanone, 1-Cyclohexyl-	5.6	126	$C_8H_{14}O$
4	Hexane, 2,3,5-Trimethyl-	10.14	128	C_9H_{20}
5	Octane, 2,4,6-Trimethyl-	13.26	156	$C_{11}H_{24}$
6	Heptadecane, 2-Methyl-	15.73	254	$C_{18}H_{38}$
7	Acetic Acid, Trichloro-, Nonyl Ester	17.70	288	$C_{11}H_{19}O_2Cl_3$
8	N-Hexadecanoic Acid	18.58	256	$C_{16}H_{32}O_2$
9	9-Tetradecen-1-Ol, (E)-	19.43	212	$C_{14}H_{28}O$
10	9,12-Octadecadienoic Acid (Z, Z)	20.18	280	$C_{18}H_{32}O_2$
11	17-Octadecynoic Acid	20.23	280	$C_{18}H_{32}O_2$
12	Octadecanoic Acid	20.43	284	$C_{18}H_{36}O_2$
13	Decane, 1-Iodo-	21.52	268	$C_{10}H_{21}I$
14	Oxalic Acid, Allyl Octadecyl Ester	22.12	382	$C_{23}H_{42}O_4$
15	Tricosane, 2-Methyl-	22.86	338	$C_{24}H_{50}$
16	Eicosane	23.15	282	$C_{20}H_{42}$
17	Eicosane, 7-Hexyl-	25.83	366	$C_{26}H_{54}$
18	Hexadecane	26.09	226	$C_{16}H_{34}$
19	Docosane, 2,21-Dimethyl-	27.24	338	$C_{24}H_{50}$
20	Tricosane, 2-Methyl-	27.54	338	$C_{24}H_{50}$
21	**26-Hydroxycholesterol**	28.89	402	$C_{27}H_{46}O_2$
22	**Lupeol**	30.99	426	$C_{30}H_{50}O$
23	5.Beta -Androstan-3. Alpha.,11.Alpha.,17.Beta.-Triol	31.54	308	$C_{19}H_{32}O_3$
24	**Beta-Sitosterol**	29.84	414	$C_{29}H_{50}O$

parameter, enabling a thorough appraisal of the powdered drug's purity and quality [36–38]. The culmination of these extensive assessments collectively contributes to the standardization process, thereby ensuring the unwavering reliability and efficacy of the herbal drug under scrutiny [36–39]. This rigorous standardization is crucial to maintain the integrity and quality of herbal medicines, thus reinforcing their credibility in pharmaceutical and healthcare applications [39].

Qualitative phytochemical analysis serves as a fundamental technique for the identification of a spectrum of bioactive compounds, encompassing alkaloids, flavonoids, tannins, terpenoids, and glycosides, embedded within plant extracts [22,23]. The comprehensive findings (Supplementary Table S3) indicate a significant presence of flavonoids in all the extracts, with relatively lower levels observed in the pet-ether extracts. Furthermore, terpenoids are uniformly evident in each of the plant extracts. Notably, the detection of fat and oils is exclusive to the ethanol and pet-ether extracts. The hydroalcoholic extract displays a notable abundance of saponins. In particular interest of the IPFAE extract, which exhibits a substantial content of carbohydrates and saponins. Additionally, the presence of sterols is discernible in the IPFE and IPFPE extracts, while alkaloids are present in the IPFAE and IPFE extracts. These comprehensive results provide valuable insights into the intricate phytochemical profiles of the extracts, underscoring their potential for diverse applications in the realm of health and wellness. The primary compound isolated from the pet-ether extract was beta-sitosterol, a prevalent sterol, utilizing column chromatography. This beta-sitosterol's purity and structural characteristics were verified through advanced instrumentation. Proton and carbon nuclei (Fig. 5a, b) were elucidated via NMR spectroscopy, functional groups were identified using FTIR, and the molecular ion peak was confirmed using GCMS, ensuring the accuracy and integrity of our findings [33–35].

The antibacterial investigation was conducted to assess the impact of *I. staphylina* flower extract on selected gram-positive and gram-negative bacterial strains, with Amikacin serving as the antibiotic standard (Tables 2–4). Impressively, all plant extracts displayed robust antibacterial activity against the targeted strains. Notably, the IPFPE extract exhibited exceptional resistance against *S. aureus* and *P. mirabilis*, resulting in zone of inhibition measurements of 16 mm at 100μg/ml and 16 mm at 50μg/ml, as well as 15 mm at 100μg/ml and 14 mm at 50μg/ml, respectively. Additionally, the ethanolic extract displayed remarkable activity against *S. aureus* and *P. aeruginosa*, yielding zone of inhibition measurements of 16 mm at 100μg/ml and 16 mm at 50μg/ml, and 15 mm at 100μg/ml and 14 mm at 50μg/mL, respectively. IPFAE also demonstrated excellent activity against *P. mirabilis* and K. *pneumonia*, with zone of inhibition measurements of 15 mm at 100μg/mL and 14 mm at 50μg/mL, and 15 mm at 100μg/mL and 14 mm at 50μg/ml, respectively. Furthermore, all extracts exhibited moderate antibacterial activity against *E. coli* and K. *pneumonia*. The efficacy of these plant extracts can be attributed to the presence of bioactive compounds that exert inhibitory effects on the growth and proliferation of a diverse range of bacterial strains, making them promising candidates for further antibacterial research and applications.

The primary aim of this research study is to conduct an in-depth investigation into the anticancer properties exhibited by ethanolic and pet-ether extracts derived from *I. staphylina* when exposed to HCT-116 cells, which serve as a prominent model for colon cancer research. To achieve this objective, we have employed the widely recognized MTT assay as our principal assessment tool. Our rationale for selecting these specific extracts is firmly rooted in their previously established exceptional antioxidant capabilities. In order to establish a comparative benchmark, we have incorporated cisplatin, a well-established standard in the field of anticancer agents, as our reference point. It is essential to emphasize that HCT-116 cells are closely associated with the development of colon cancer in humans, rendering them a highly relevant model for our investigative endeavors. Our research outcomes have unequivocally revealed a substantial reduction in cell viability (Figs. 3, 4 and Tables 5–7) subsequent to exposure to the pet-ether and methanolic extracts of *I. staphylina*. This pronounced inhibition can be attributed to the presence of secondary metabolites and antioxidants within the samples, leading to the aggregation and deformation of proteins within the cancer cells, ultimately inducing apoptosis [40–48]. These findings strongly suggest that IPFPE and IPFE hold the potential to induce apoptosis and inhibit cellular proliferation in HCT-116 cells, thereby presenting a promising avenue for the prevention of colon cancer [43–45]. Moreover, our MTT assay results have demonstrated a gradual reduction in cell viability with increasing concentration. Notably, at concentrations of 51.44 μM (IPFE) and 52.74 μM (IPFPE), we observed a 50 % growth inhibition, underscoring their robust pro-apoptotic activity against the HCT-116 cancer cell line. Several herbs and phytochemicals have been scientifically proven to possess cytoprotective effects using the MTT test [46–50]. Inducing apoptosis in cancer cells is a well-established strategy for the development of anticancer drugs [47, 48]. Numerous plant-derived substances have been investigated for their ability to trigger apoptosis, consistent with previous studies [43–52]. These results lend strong support to the potential of *I. staphylina* flower extract as a valuable source of chemotherapeutic agents, given the prioritization of apoptosis in anticancer drug development. While these findings are promising, further extensive research is warranted to solidify this assertion.

3. Conclusion

This study centered on the relatively uncommon *I. staphylina* flower, with the primary aim of furnishing valuable scientific evidence for its traditional usage. Through successful extraction and subsequent pharmacognostic evaluations, we endeavored to elucidate its potential pharmacological applications. Guided by ethnopharmacological insights, we explored the flower extract's efficacy against selected bacterial strains and its inhibitory effects on HCT-116 cancer cells. The findings demonstrate significant antibacterial activity and notable inhibition of HCT-116 cancer cells. Furthermore, the isolation of pure beta-sitosterol from the pet-ether extract was achieved. These results strongly suggest that the *I. staphylina* flower extract may represent a

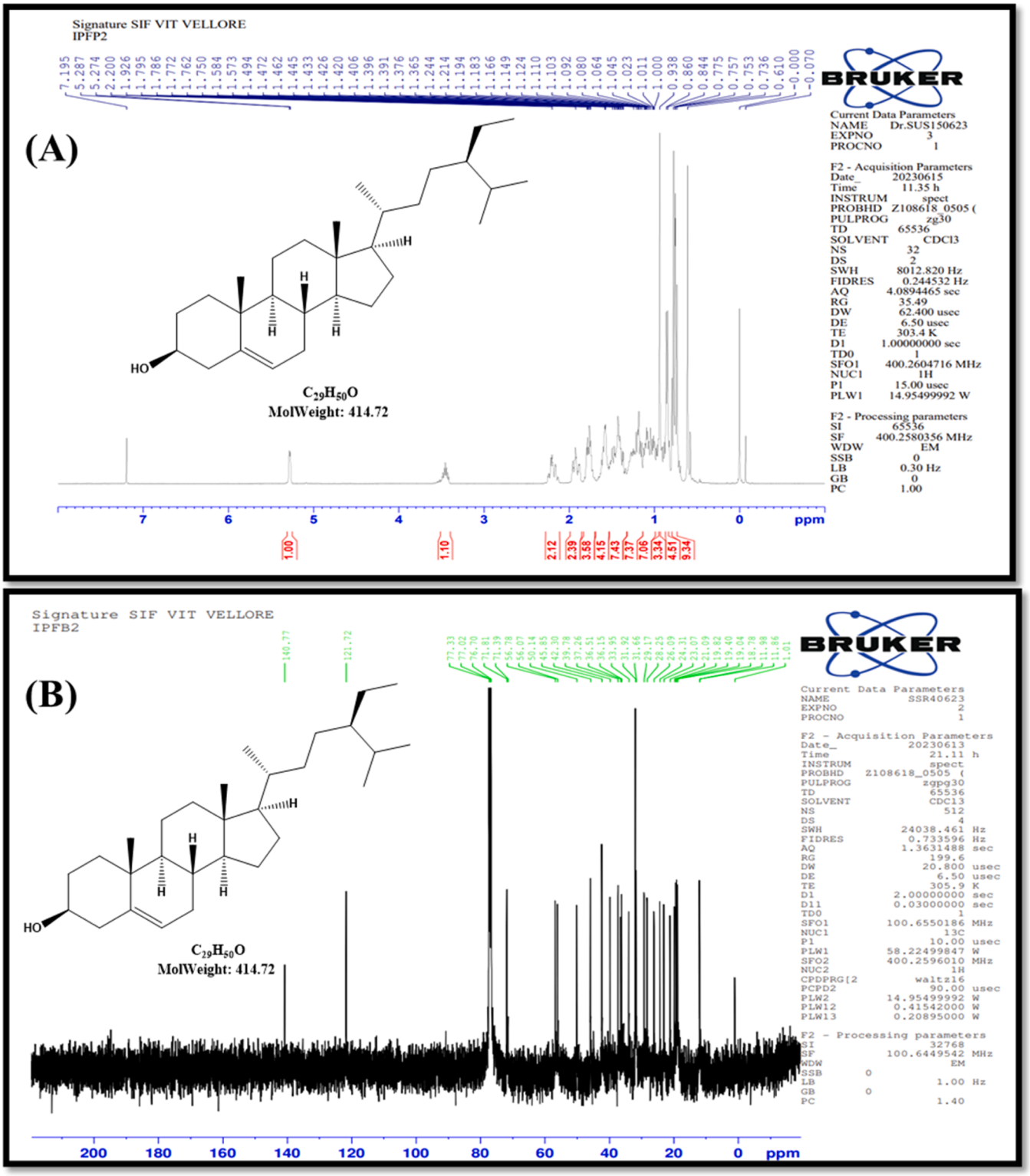

Fig. 5. (**a**) ^{1}H NMR of isolated compound, (**b**) ^{13}C NMR spectrum isolated compound.

promising reservoir for the development of novel natural antibiotics and anticancer therapeutics. Such discoveries offer auspicious prospects for advancing both healthcare and the field of biological sciences.

Code expansions

IPFPE, which stands for the *I. staphylina* flower pet-ether extract; IPFE, representing the *I. staphylina* flower ethanolic extract; and IPFAE, indicating the *I. staphylina* flower aqueous extract.

CRediT authorship contribution statement

Lakshmanan Narayanan: Writing – original draft, Formal analysis, Conceptualization. **T. Maruthavanan:** Data curation, Resources. **R. Vaithiyanathan:** Formal analysis, Resources, Writing – review & editing. **Geetha Venkatesan:** Data curation. **K.E. Vivekanandan:** Data

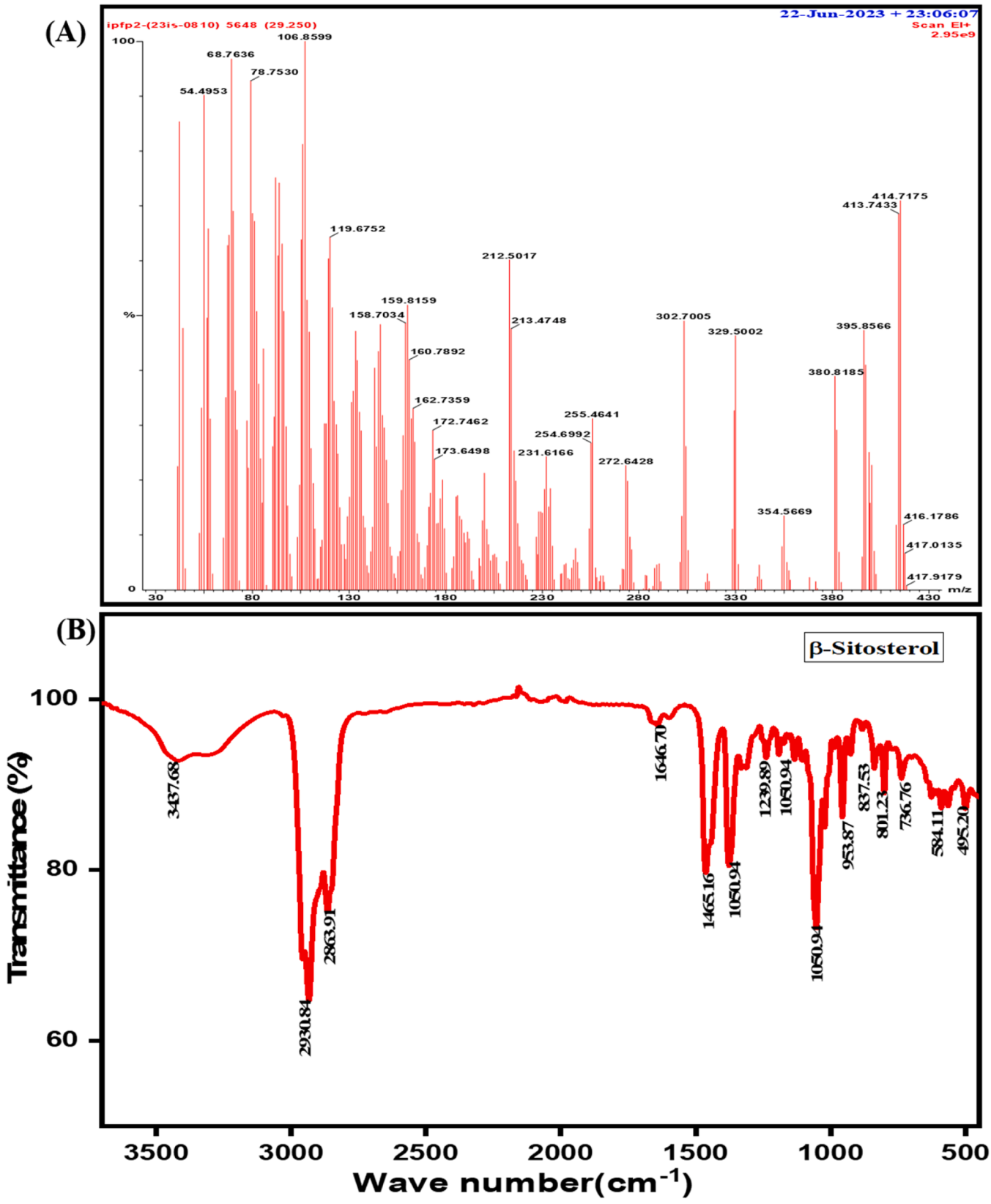

Fig. 6. (**a**) GCMS spectroscopy of isolated compound and (**b**) FTIR analysis of isolated compound.

curation. **Gayathri Kaliyannan:** Resources. **Paskalis Sahaya Murphin Kumar:** Writing – review & editing, Supervision, Project administration. **Mary Sahaya Anisha John Bosco:** Visualization, Validation, Software. **Govindhasamy Murugadoss:** Writing – review & editing, Project administration. **S.R. Suseem:** Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2025.142045.

Data availability

Data will be made available on request.

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