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Phytochemical Profiling by GC–MS and Evaluation of the Anticancer, Antioxidant, and Anti-Inflammatory Potential of *Solanum surattense*

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Abstract
Solanum surattense (*S. surattense*) is a member of the genus *Solanum* and family *Solanaceae*, which comprises approximately 99 genera and 2000 species. The present work was conducted to evaluate GC-MS and FTIR analyses, followed by biological activity assessment of the medicinal plant *S. surattense* from Baluchistan. Pakistan. The GC-MS was used to identify volatile constituents present in the n-hexane fraction of the plant. The functional groups of these were identified in the methanolic extract through FTIR analysis. Moreover, anti-inflammatory, antioxidant, and anti-cancer properties were evaluated in the plant's methanolic extract using colorimetric, free-radical scavenging, and chemiluminescence methods, respectively. Twenty-five volatile compounds were identified by GC-MS analysis. The FTIR spectrum of the methanolic extract also indicated the presence of various functional groups, including alcohols, alkanes, aldehydes, aromatic compounds, and esters.The anticancer activity results demonstrated that the methanolic extract exhibited a relatively high IC_{50} value of 90.5 ± 1.5 µg/mL. In contrast, the standard drug Doxorubicin exhibited a much lower IC_{50} value of 0.79 ± 0.13 µg/mL. The methanolic extract of the plant exhibited minimal cytotoxic potential compared with HeLa cervical cell line. The antioxidant activity results revealed that the methanolic extract of the plant exhibited inhibition with an IC_{50} of 800 ± 5 µg/mL, indicating weak antioxidant activity compared to the standard antioxidant, gallic acid, which had an IC_{50} of 21.7 ± 0.22 µg/mL. These findings suggest that *Solanum surattense* possesses antioxidant potential, although its efficacy is considerably lower than that of gallic acid. The methanolic extract of *S. surattense* showed moderate anti-inflammatory activity (66.6 ± 13.2 µg/mL) compared with ibuprofen (11.2 ± 1.9 µg/mL). Despite its lower potency, the plant demonstrated promising anti-inflammatory properties and may serve as a potential natural source of anti-inflammatory compounds.

Keywords: GC-MS, FTIR, Antioxidant, Anti-inflammatory, Anticancer

1. Introduction
Medicinal plants are well known as one of the earliest sources of therapeutic remedies and have been used for centuries in traditional healthcare systems used in various parts of the world. Observations and experience have accumulated knowledge of their medicinal properties, and human society has transmitted it from generation to generation [1].Natural products remain a valuable source of therapeutic agents, and several modern medicines derived from traditional herbal medicines are now routinely used in pharmacology [2].The *Solanaceae family* comprises about 2,300 species of herbs, shrubs, and small trees and isa rich source of bioactive compounds of therapeutic significance in humans [3]. *S.surattense*, also known as *S.xanthocarpum*, and commonly referred to as Kantakari, Kandiari. They are widely distributed in Pakistan, India, Bangladesh, North Africa, Australia, and Polynesia. In Pakistan, the plant commonly grows in dry and semi-arid regions, along roadsides, wastelands, and cultivated fields, particularly in Balochistan and Khyber Pakhtunkhwa [4].
The plant is a prickly, diffuse herb with bright yellow berries and violet-blue flowers. Its stems and leaves are covered with sharp prickles, while the fruits are spherical in shape, initially green when immature and turning yellow upon ripening [5]. *S.surattense* possesses a wide range of pharmacological activities. Different species have been reported to possess immunomodulatory, cardioprotective, antihyperglycemic, antioxidant, antimicrobial, anti-asthmatic, hepatoprotective, anticancer, analgesic, molluscicidal, antifungal, and antidepressant properties [6, 7].
The fruit paste of *S. suranttense* is used externally to treat inflammation, skin eruptions, and pimples [8]. Additionally, a paste prepared from the roots, mixed with lemon juice, is used by traditional healers to treat snakebites. Further, the plant is said to be antipyretic, a fat burner, and a natural blood purifier in nature [9]. It has also been used to treat the common cold, intestinal worms, and sleep disorders [10]. Moreover, the plant has been reported to have laxative action and to be used for enlarging the liver and for aphrodisiac activities [11, 12]. The stem, flowers, and fruits of S. surattense are used in traditional medicine to treat burning sensations of the feet with the presence of vesicles. Its anti-diabetic property has also been validated in experimental studies in diabetic animal models [13, 14]. In addition, the warm aqueous extract of dried fruits is widely used to treat fever, cough, and certain cardiovascular diseases [15].
S. *suranttense* has been widely reported for its ethno-medicinal applications. Previous studies have documented its phytochemical constituents and various biological activities. The present study focused on evaluating plant samples collected from Balochistan. The research primarily involved characterizing volatile constituents using GC-MS, while the plant's methanolic extract was assessed for antioxidant, anticancer, and anti-inflammatory activities.

2. Materials and Methods

2.1 Collection and identification of Plant

The plant material was collected from Dahna-sar, located in the Koh-e-Suleiman Range, northern Balochistan, Pakistan. It was identified by Dr. Ayesha Masood, Assistant Professor, Department of Botany, University of Balochistan, Quetta. The specimen was submitted to the Herbarium record under voucher number UOB-000645.

2.2 Extraction

The plant material (5 kg) was washed, shade-dried, and then ground into fine powder. The powdered material was soaked in 10 L of methanol at room temperature for 7 days, and the process was repeated 3 times. The methanolic extract was filtered through Whatman filter paper and condensed under reduced pressure in a rotary evaporator at 45 °C. The concentrated crude extract was freeze-dried at 4 °C, resulting in a gummy residue.

2.3 Fractionation

The crude methanolic extract was homogenized in distilled water to get an aqueous solution. The aqueous extract was mixed with n-hexane in a separating funnel to separate the n-hexane fraction.

2.4 GC-MS Analysis

The phytochemical constituent was analyzed by using GCMS. The instrument is an HP-5MS capillary column with a stationary phase of 5% phenyl methyl siloxane. Helium is used as a carrier gas at a flow rate of 1 mL/min and to control the pressure. The oven temperature was adjusted between 60 and 280 °C. Identification of the detected compounds was accomplished by the NIST spectral library [16].

2.5. FTIR Analysis

FTIR analysis was employed to determine the functional groups present in different compounds. The spectra were obtained over the range 4000–400 cm^{-1} with 4 cm^{-1} resolution and 32 scans, using a deuterated triglycine sulfate detector at room temperature. Absorption peaks were used to identify functional groups [17].

2.6. Biological Activities

2.6.1 Anticancer activity

Anticancer activity of the plant extract was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, HeLa cells (Cervical Cancer) were cultured in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin, and 100 µg/ml of streptomycin in 75 cm2 flasks, and kept in 5% CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted using a hemocytometer, and diluted in a specific medium. Cell culture with a concentration of 6×10^{-4} cells/ml was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, the medium was removed, and 200 µL of fresh medium was added with different concentrations of compounds (1-30µM). After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring absorbance at 570 nm using a microplate reader (Spectra Max Plus, Molecular Devices, CA, USA). Cytotoxicity was assessed as the concentration that caused 50% growth inhibition (IC50) in HeLa cells. The percent inhibition was calculated by using the following formula:

% inhibition = 100-(mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100).

The results (% inhibition) were processed using SoftMax Pro software (Molecular Devices, USA) [18,19].

2.6.2 Antioxidant activity

DPPH solution (95 µl, 300 µM) in ethanol was mixed with the test solution (5 µl, 500 µM). The reaction was allowed to proceed for 30 min at 37 °C, and absorbance was monitored using a multiplate reader (SpectraMax340) at 517 nm. Upon reduction, the color of the solution fades (Violet to pale yellow). Percent Radical Scavenging Activity (%RSA) is determined by comparison with a DMSO-containing control. The concentration that reduces the initial DPPH concentration by 50% is defined as the IC50. The IC_{50} values of compounds were calculated by using the EZ-Fit Enzyme kinetics software program (Perrella Scientific Inc., Amherst, MA, USA). N-acetylcysteine, ascorbic acid, and BHA are used as the referencecompounds[20].

2.6.3 Anti-inflammatory activity

A luminol-enhanced chemiluminescence assay was performed to assess anti-inflammatory activity of methanolic extract of the plant. Briefly, 25 µL of diluted whole blood HBSS++ (Hanks Balanced Salt Solution, containing calcium chloride and magnesium chloride) [Sigma, St. Louis, USA] was incubated with 25 µL of three different concentrations of compounds (1, 10, and 100 µg/mL), each in triplicate. Control wells received $HBSS^{++}$ and cells, but no compounds. Test was performed in white half area 96 well plates [Costar, NY, USA], which was incubated at 37 ºC for 15 minutes in the thermostat chamber of luminometer. After incubation, 25 µL of serum opsonized zymosan (SOZ) and 25 µL of intracellular reactive oxygen species detecting probe, luminal were added into each well, except blank wells (containing only $HBSS^{++}$). The level of the ROS was recorded in luminometer in term of relative light units (RLU)[21].

3.0 Results and Discussions

3.1 GC-MS Analysis

The GC-MS analysis was observed in the n-hexane fraction of Solanum surattense. The findings of the current investigation revealed the existence of 25 naturally occurring compounds, including arachidic acid, palmitic acid, stearic acid, myristic acid, oleic acid, and cis-vaccenic acid, cis-11-eicosenoic acid, 9,12-octadecadienoic acid, elaidic acid. These fatty acid derivatives play important biological and physicochemical roles [22]. Capric acid and lauric acid are act as antimicrobial properties, while linoleic acid is an essential fatty acid required for various physiological functions, including maintenance of skin health and immune system regulation [23].β-D-Glucopyranose, 1,6-anhydro, identified as a sugar derivative, functions as a metabolic intermediate, an environmental tracer, and a bio-based chemical building block [24]. The compounds such as Z-13-octadecenal, 9,17-octadecadienal, (6Z,9Z)-pentadecadienal and 2-hydroxycyclopentadecanone belong to the carbonyl group compounds, mainly aldehydes and ketones, and possess significant biological activities, particularly antimicrobial and other bioactive properties [25]. In addition, indole and delta-thionodecalactone were identified as important bioactive compounds exhibiting anticancer, anti-inflammatory, and neuroactive properties [26]. Other compounds, such as squalene, γ-tocopherol, and stigmasta-7,25-dien-3-ol, are widely known for their diverse biological and pharmacological activities [27].The identified compounds are presented in Table 1, while the chromatogram and mass spectra of the volatile constituents are illustrated in Figures 1 and 2 (supplementary file), respectively.

Table.1: List of volatile compounds identified by GC-MS analysis

S.No.	Name of Compound	Area Sum	Retention Time	Molecular Weight	Molecular Formula
1	Capricacid	0.01	6.021	172.27	$C_{10}H_{20}O_2$
2	Arachidicacid	0.18	6.178	304.47	$C_{20}H_{32}O_2$
3	β-*D*-Glucopyranose, 1,6-Anhydro	0.04	6.897	162.14	$C_6H_{10}O_5$
4	Palmiticacid	0.01	7.289	256.42	$C_{16}H_{32}O_2$
5	Lauricacid	0.02	7.320	200.32	$C_{12}H_{24}O_2$
6	Stearic acid	0.02	7.587	284.48	$C_{18}H_{36}O_2$
7	Myristic acid	0.09	7.978	228.38	$C_{14}H_{28}O_2$
8	n-Hexadecanoicacid	0.03	12.999	256.32	$C_{16}H_{32}O_2$
9	Estra-1,3,5(10)trienebetaol	0.06	14.135	256.38	$C_{18}H_{24}O$
10	Octadecanoicacid	0.01	14.564	284.48	$C_{18}H_{34}O_2$
11	Cis-vaccenicacid	0.06	15.011	284.48	$C_{18}H_{34}O_2$
12	Oleicacid	0.02	15.253	294.48	$C_{18}H_{34}O_2$
13	Cis-11-Eicosenoicacid	0.04	16.032	310.51	$C_{20}H_{38}O_2$
14	13-Octadecenal(Z)	0.04	16.624	266.41	$C_{18}H_{34}O$
15	Cyclopentadecanone,2-hydroxy	0.19	18.395	240.38	$C_{15}H_{28}O_2$
16	9,12-Octa decadienoicacid	0.06	18.987	280.45	$C_{18}H_{32}O_2$
17	Elaidicacid	0.09	19.476	282.38	$C_{18}H_{34}O_2$
18	Indole	0.04	19.923	117.15	C_8H_7N
19	9,17-octadecadienal,Z	0.38	21.349	264.32	$C_{18}H_{32}O$
20	Squalene	1.71	22.274	410.73	$C_{30}H_{50}$
21	(6Z,9Z)-pentadecadienal	0.14	22.534	222.37	$C_{15}H_{26}O$
22	Δ-thionodecalactone	0.01	23.663	170.25	$C_{10}H_{18}O_2$
23	γ-Tocopherol	4.58	24.189	416.7	$C_{28}H_{48}O_2$
25	Stigmasta-7,25-dien3-ol,(3-β,5-α)	3.12	26.261	412.6	$C_{29}H_{48}O$

These volatile constituents were classified according to their chemical structures and functional groups, including terpenoids and steroids (9.99%), carbonyl compounds such as aldehydes and ketones (0.75%), fatty acid derivatives (0.64%), sugar derivatives (0.15%) and other miscellaneous compounds (88.47%).

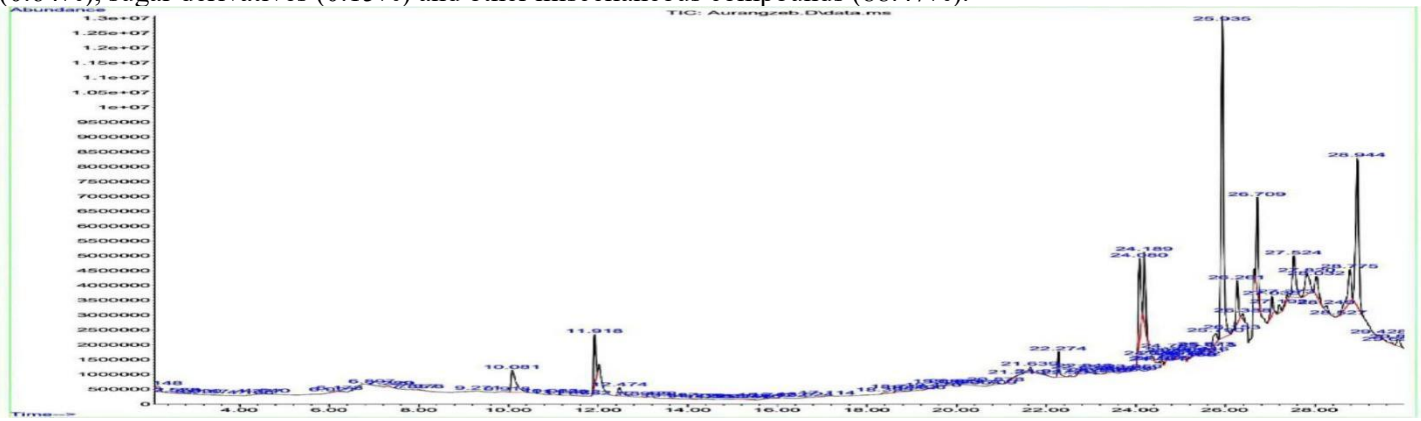

Figure 1. GC-MS Chromatogram of n-hexane fraction of *Solanum surratense*

Figure 2. Mass spectra of volatile compounds **(See Supplimentary file)**

3.2. FTIR Analysis

FTIR analysis was observed to determine the functional group present in the methanolic extract of plant. FTIR spectrum revealed the presence of alcohols, alkanes, aldehyde, aromatic compounds and esters. The presence of these functional groups indicates the complex phytochemical nature of the methanolic extract. The findings are described in Table 2, while the IR spectrum of the methanolic extract is reported in Figure 3.

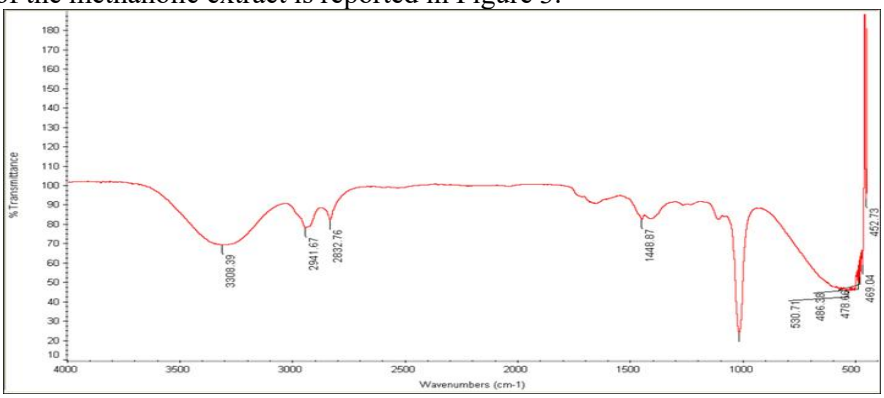

Figure.3.FTIR spectra of methanolic extract obtained from medicinal plant *Solanum surratense*

A wide absorption peak at 3308.39cm^{-1} was observed, which was due to the hydroxyl stretching vibration, indicate, alcohol and phenolic compounds. The peaks observed at 2941.67 cm^{-1} and 2832.76 cm^{-1} were related to the vibrations of aliphatic C–H bonds of alkanes and aldehydic groups, respectively.Moreover, the absorption band at 1448.87 cm-1 was attributed to aromatic C=C bond bending and the sharp band at 1019.5 cm^{-1} to C–O stretching in ester or glycosidic bond.

Table.2. List of functional groups determined through FTIR analysis

Methanolic extract	Functional Group	General formula	Vibrational modes
3308.39	Alcohol	O-H	Stretching
2941.67	Alkanes	C-H	Asymmetric stretching
2832.76	Aldehyde	RCHO	Symmetric stretching
1448.87	Aromatic Alkene	C=C	Bending
1019.5	Ester	RCOOR	Stretching

3.3. Biological activities

3.3.1 Anticancer activity

The anticancer activity was determined by MTT assay using the HeLa cell line. The findings showed the methanolic extract was found to have a percentage inhibition of (66.4%) at 200 µg/mL, but a very high IC_{50} value (90.5 ± 1.5 µg/mL) in comparison to standard Doxorubicin (0.79 ± 0.13 µg/mL), signifying high potency of inhibition (83.71%). This indicates the plant extract has shown low cytotoxic potential against the HeLa cervical cell line; its efficacy was much lower than the standards. The results are described in Table 3 and Figure 4.

Table.3. Anticancer potential of *Solanum surattense*

Sample	Concentration µg/mL	Inhibition%	IC_{50} ± SD
Methanolic extract	200µg/ml	66.4	90.5±1.5
Doxorubicin	200µg/ml	83.71	0.79±0.13

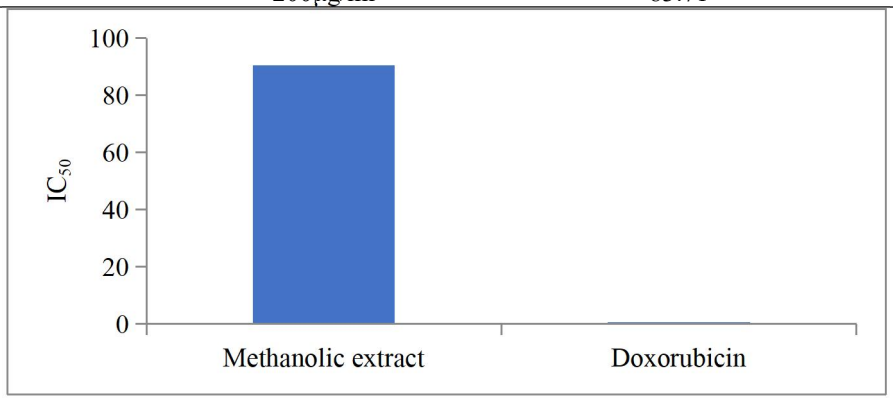

Figure.4. Comparative evaluation of the methanolic extract and Doxorubicin

3.3.2 Antioxidant activity

The antioxidant activity of the methanolic extract of *S.surattense* was evaluated at various concentrations and compared with the known antioxidant compound gallic acid. The methanolic extract exhibited a concentration-dependent free radical scavenging activity, showing 85.46% inhibition at 2250 µg/mL, 65.6% at 1125 µg/mL, 40.79% at 562.5 µg/mL, 11.26% at 140 µg/mL, 11.0% at 70 µg/mL, and 5.19% at 35 µg/mL. These results indicated that the antioxidant potential of the methanolic extract increased with increasing concentration, as evidenced by the progressive rise in percentage

inhibition of free radicals.The IC_{50} value of the methanolic extract was determined as 800 ± 5 µg/mL, which is the dose needed to scavenge 50% of free radicals. The radical scavenging activity (RSA) classification of the extract was "weakly active," indicating moderate antioxidant activity.The result revealed that the standard antioxidant, gallic acid, achieved 95.3% inhibition at 500 µg/mL and a significantly lower IC_{50} value of 21.7 ± 0.22 µg/mL compared with the other antioxidants. Gallic acid was classified as strongly active by using the RSA classification.Overall, the antioxidant activity of the methanolic extract of *Solanum surattense* was shown to be concentration dependent, although it was much lower than the standard antioxidant gallic acid, evidenced by the higher IC_{50} value and lower radical scavenging activity.The findings are described in Table 4 and Figure 5.

Table 4. Anti-oxidant potential of *S.surattense*

Sample	Concentration µg/mL	Inhibition%	IC_{50} ≈ value	RSA
Methanolic extract	2250µg/ml	85.46	800±5	Weak active
	1125 µg/ml	65.6		
	562.5 µg/ml	40.79		
	140 µg/ml	11.26		
	70 µg/ml	11		
	35 µg/ml	5.19		
Gallic Acid	500µg/ml	95.3	21.7±0.22	Strong active

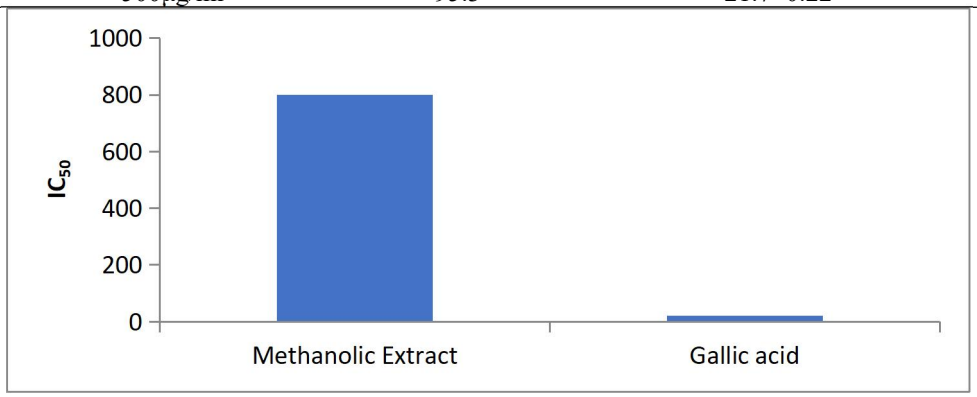

Figure 5. Comparative evaluation of the methanolic extract and Gallic acid

3.3.3 Anti-inflammatory Activity

Anti-inflammatory activity of *S.surattense* was assessed by using various concentrations of the methanolic extract and was compared with the standard anti-inflammatory drug, ibuprofen. The methanolic extract was found to be concentration-dependent, showing 103.4% inhibition at 250 µg/mL, 38.4% at 50 µg/mL, and -5.4% at 10 µg/mL. The negative inhibition value at the lowest concentration indicates that there was no anti-inflammatory activity at this concentration. The IC_{50} value of the extract was 66.6 ± 13.2 µg/mL, indicating the concentration required to inhibit 50% of the activity. In contrast, ibuprofen showed a higher inhibition percentage (73.2%) at 25 µg/mL and a lower IC_{50}value (11.2 ± 1.9 µg/mL) which indicates its greater anti-inflammatory activity. Overall, the results suggest that the methanolic extract of *Solanum surattense* possesses significant anti-inflammatory activity, although its effectiveness is lower than that of the standard drug ibuprofen. The findings are displayed in Table 5 and Figure 6.

Table 5 Anti-inflammatory potential of *S.surattense*

Sample	Conc-(µg/ml/µm)	%Inhibition	IC50± SD µg/ml
Methanolic extract	250 µg/ml	103.4	66.6±13.2
	50 µg/ml	38.4	
	10 µg/ml	-5.4	
Ibuprofen	25 µg/ml	73.2	11.2±1.9

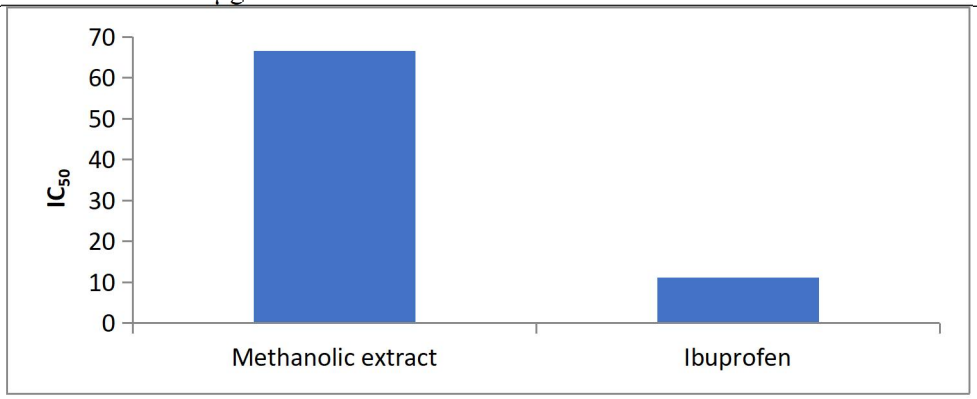

Figure 6. Comparative anti-inflammatory evaluation of the methanolic extract and Ibuprofen

4.0 Conclusion

This study investigated the phytochemical composition and biological activities of *Solanum surattense* collected from Balochistan. GC–MS analysis identified 25 volatile constituents, predominantly miscellaneous compounds (88.47%), along with terpenoids and steroids (9.99%), carbonyl compounds (0.75%), fatty acid derivatives (0.64%), and sugar derivatives (0.15%). FTIR analysis confirmed the presence of alcohols, alkanes, aldehydes, aromatic compounds, and esters. The methanolic extract exhibited moderate anticancer activity against the HeLa cervical cancer cell line, with 66.4% inhibition at 200 µg/mL and an IC_{50} of 90.5 ± 1.5 µg/mL, which was considerably lower than that of doxorubicin. The extract also exhibited antioxidant activity, although its potency was lower than that of gallic acid, with an IC_{50} of 800 ± 5 µg/mL. Significant concentration-dependent anti-inflammatory activity was observed, with 103.4% inhibition at 250 µg/mL and an IC_{50} value of 66.6 ± 13.2 µg/mL, though its efficacy remained lower than that of ibuprofen. Overall, *S. surattense* contains bioactive phytochemicals with antioxidant, anticancer, and anti-inflammatory properties, supporting its ethnomedicinal use. However, the observed activities were modest compared with standard drugs, highlighting the need for further studies on the isolation, characterization, and pharmacological evaluation of its active constituents.

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Author's Contribution:

Aurangzeb Ashraf: Conceptualization, investigation, methodology, formal analysis, data curation, validation, writing original draft. Samar Ali: Supervision, review, validation, investigation, data curation. Nimra Fazal: Formal analysis, validation, literature search, data curation. Syed Shabeer Ahmed: Visualization. Sara Zameer: Editing. Nizam Ud-Din Baloch: Facilitation and resources.

Conflict of Interest: The authors declare no conflict of interest

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