Received: 16 November 2024 | Revised: 26 January 2024 | Accepted: 28 February 2025
DOI: https://doi.org/10.69547/TSFJB.030104

TSF JOURNAL OF BIOLOGY

EISSN: 3006-0478
PISSN: 3006-0869

Website link: www.tsfjb.com

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To cite: Ali R, Anwar MA, Jan SA. Phytochemical Composition and Biological Properties of *Berberis lycium* and *Solanum surattense*. TSF Journal of Biology.2025; 3(1):40-48.

Article QR

Published online: 17 March 2025
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Research Article

Phytochemical Composition and Biological Properties of *Berberis lycium* and *Solanum surattense*

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Keywords:
Antioxidants, Anticancer, *Berberis lycium*, Prostate cancer, *Solanum surattense*

ABSTRACT

The present study was designed to assess the potential health benefits of *Berberis lycium* and *Solanum surattense* by evaluating their phytochemical content, and their biological activities, including antioxidant and anticancer potential. The antioxidant capacity of the extracts was assessed by using the DPPH radical scavenging assay, with IC_{50} values calculated to compare their relative antioxidant strength. The MTT assay measured cytotoxicity towards human prostate cancer cells (PC3). *B. lycium* exhibited significantly higher total phenolic content (193.83 ± 3.93 mg GAE/g) and total flavonoid content (37.47 ± 0.37 mg QE/g) compared to *S. surattense* (50.69 ± 3.53 mg GAE/g and 19.41 ± 0.33 mg QE/g, respectively), suggesting a rich profile of antioxidant compounds in *B. lycium*. Consistent with these findings, *B. lycium* extract exhibited significantly stronger DPPH radical scavenging activity (IC_{50} = 4.10 ± 0.06 µg/mL) followed by *S. surattense* extract (IC_{50} = 4.43 ± 0.11 µg/mL). Therefore, *B. lycium* could act as a potential source of antioxidant compounds for isolating new antioxidant molecules through bioassay-guided fractionation. Cytotoxic evaluation of *B. lycium* and *S. surattense* against human prostate cancer cells (PC3) was performed using the MTT assay. Results revealed significant cytotoxic effect of *S. surattense* (IC_{50} = 42.01±1.31µg/mL) compared with the standard drug Doxorubicin (IC_{50} = 14.13±2.08µg/mL). *Berberis lycium* showed weak cytotoxic effect (IC_{50} = 309.06±3.55 µg/mL). The results demonstrated an active anti-proliferative role of *S. surattense* extracts on human prostate cancer cells which suggests the use of this plant as a novel targeted therapy in human prostate cancer.

1. INTRODUCTION

For centuries, medicinal plants have been used to combat various infectious diseases and serve as a source of lead molecules for drug development. Pakistan hosts diverse medicinal flora, with approximately 600 documented medicinal plant species traditionally used to treat various ailments, many of which remain understudied [1]. Plants produce a diverse range of phytochemicals with therapeutic potential, including antioxidant, anti-inflammatory, and anticancer properties. Flavonoids (such as quercetin, rutin, and anthocyanins), carotenoids (like beta-carotene, lycopene, and lutein), polyphenols (including resveratrol, curcumin, and catechins), alkaloids (such as morphine, caffeine, and nicotine), terpenes (including limonene, menthol, and camphor), saponins, and coumarins are among the most studied phytochemicals [2].

Berberis lycium, a key medicinal plant, belongs to the *Berberidaceae* family. It has a wide geographical distribution across America, Asia, Europe, and Africa, thriving in the mountainous regions of Pakistan [3]. Locally, it is known as *"Kashmal"* or *"Kasmal"* in Urdu and by other common names, including English barberry, Indian barberry, and Indian lycium. Each portion of *B. lycium* has significant medicinal value [4]. The reported bioactive compounds isolated from *B. lycium* include umbellatine, punjabine, sindamine, chinabine, gilgitine, jhelumine, baluchistanamine, berberine, palmitine, and berbamine. Among them, berberine is one of the key metabolites with multiple activities against different types of diseases [5].

Solanum surattense is one of the notable species of *Solanaceae*, which comprises approximately 2,300 species of diverse plant types. It is predominantly found in Southeast Asian regions, including Malaysia, Ceylon, Australia, Pakistan, and India [6]. *S. surattense* possesses important secondary metabolites with potent biological activities [7]. However, limited studies are available on the anticancer and antioxidant properties of *B. lycium* and *S. surattense* from Pakistan. This study explores the potential health benefits of *B. lycium* and *S. surattense* by evaluating their biological activities, including anticancer properties.

2. METHODOLOGY

2.1 Plant Collection, Sample Preparation and Extraction

B. lycium and *S. surattense* were collected in May 2024 from the fields of Kotli Sattian district, Rawalpindi, Pakistan, and were shade-dried at room temperature (28°C±3) for 15 days. The roots of *B. lycium* and the aerial parts of *S. surattense* were used for analysis. After proper drying, the plant material was ground into a fine powder and stored at -20°C. Samples of *S. surattense* (27.22 g) and *B. lycium* (49.24 g) were macerated in methanol under ambient conditions for 72 hours. The extracts were strained, concentrated under reduced pressure in a rotary evaporator, and stored at -20°C.

2.2 Quantification of Total Phenolic Content (TPC)

TPC in both plant extracts was quantified using the Folin-Ciocalteu (FC) reagent method [8]. An aliquot of diluted plant extract (100 µL) was mixed with FC reagent (diluted 10X with distilled water) and a 6% sodium carbonate solution. The resultant colored solution was measured spectrophotometrically at 725 nm. A calibration curve generated using gallic acid was used to calculate the TPC and were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract (mg GAE/g).

2.3 Quantification of Total Flavonoid Content (TFC)

TFC content of the *B. lycium* and *S. surattense* extracts was assessed via a modified aluminum chloride colorimetric assay [8]. Extracts (1 mg/mL in DMSO) were mixed with a pre-mixed solution of methanol, 10% aluminum chloride, 1M potassium acetate, and water. The absorbance of the resulting colored complexes was determined at 415 nm on spectrophotometer. A standard curve of quercetin was formulated to determine the TFC and were expressed as milligrams of quercetin equivalents (QE) per gram of dry extract (mg QE/g).

2.4 DPPH Radical Scavenging Assay

The antioxidant potential of *B. lycium* and *S. surattense* extracts was assessed via the DPPH radical scavenging assay [9]. The standard solution of DPPH radical (60 µM) was prepared in Methanol and diluted to an absorbance of approximately 0.7 at 517 nm**.** Various concentrations (1, 5, and 50 µg/mL) of plant extracts were mixed with the DPPH radical

$$Scavenging\ (\%) = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\ x100$$

solution, placed in the dark for 30 minutes, and then absorbance at 517 nm was determined on spectrophotometer. Ascorbic acid was used as a reference standard for comparative analysis. The scavenging (%) of DPPH radical by plant extracts was computed using the following formula:

2.5 Anticancer Activity

MTT assay was employed to determine the anticancer activity of *B. lycium* and *S. surattense* extracts against human prostate cancer cells (PC3) [10]. PC3 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) augmented with the fetal bovine serum (FBS), streptomycin, and penicillin. The PC3 cells were grown in CO_2 incubator at 37°C with 5% CO_2. Subsequently, the PC3 cells were incubated with different concentrations (7.5-30 µg/mL) of plant extracts for 48 hours. Doxorubicin was used as a positive control and Dimethyl sulfoxide (DMSO) was used as a negative control. The percentage of growth inhibition was calculated using the following formula:

Inhibition (%) = 100 - [(Mean O.D of Sample - Mean O.D of Negative Control) / (Mean O.D of Positive Control - Mean O.D of Negative Control)] * 100

2.6 Statistical Analysis

Regression analysis was done on Microsoft Excel to calculate IC_{50} values of plant extracts in biological activities. Data from three replicates of each experiment were calculated and reported as the mean ± standard deviation. The analysis of variance (ANOVA) and Tukey's post hoc test were conducted using Minitab 18 software to calculate significant difference among the tested groups.

3. RESULTS

3.1 Total phenolic Content, Total Flavonoid Content and Extraction Yield of *Berberis lyceum* and Solanum surattense

In the present study, *B. lycium* exhibited higher TPC (193.83 ± 3.93 mg GAE/g) and total flavonoid content (37.47 ± 0.37 mg QE/g) compared to *S. surattense* (50.69 ± 3.53 mg GAE/g and 19.41 ± 0.33 mg QE/g, respectively). The following results suggested that *B. lycium* may be a richer source of antioxidant compounds compared to *S. surattense*, potentially due to specific biosynthetic pathways or environmental factors. The extraction yield (%) for both plants is presented in Table 1.

3.2 Antioxidant Activity

DPPH radical scavenging activity of *B. lycium* and *S. surattense* methanol extracts was evaluated at different concentrations (1, 5, 50 µg/mL) to minimize the assay noise and amplify the effect of the extracts on the DPPH radical. The decrease in the absorbance was observed with the increasing concentration of the extracts in the reaction mixture. The percentage of DPPH radical scavenging of both extracts increased in a dose-dependent manner (Table 2). Both plants showed excellent antioxidant activity comparable to ascorbic acid. According to Table 2, ascorbic acid displayed the strongest free radical scavenging activity, followed by *B. lycium* and *S. surattense* extract. These findings suggest that *B. lycium* and *S. surattense* extracts possess significant antioxidant potential, which could be used for the isolation of potential therapeutic compounds.

3.3 Anticancer Activity

The results of the anticancer activity of *B. lycium* and *S. surattense* extracts against PC3 cells are shown in Table 3. Interestingly, *S. surattense* extract exhibited higher inhibition than that of *B. lycium* despite lower TPC and TFC. This condition suggests that *S. surattense* may contain phytochemicals other than phenolics and flavonoids that are responsible for its cytotoxicity. These findings indicate that the crude extracts have anti-cancer properties. Therefore, additional investigations are required to elucidate the precise nature of the bioactive components responsible for the observed cytotoxic effects.

Table 1: Total phenolic content, total flavonoid content and extraction yield of *Berberis lycium* and *Solanum surattense*

Plant	TPC (mg GAE/g)	TFC (mg QE/g)	Extraction Yield (%)
Berberis lycium	193.83±3.93a	37.47±0.37a	3.43
Solanum surattense	50.69±3.53b	19.41±0.33b	3.52

Note: Each value in the table is expressed as mean ± SD (n=3), Means not sharing the same letter are significantly different at the $P < 0.05$ probability level in each column.

Table 2: Percentage of DPPH Radical Scavenging by Methanol Extracts of *B. lycium* and *S. surattense* and their antioxidant effect (IC_{50}) at various concentrations

Plant	DPPH Radical Scavenging (%)			IC_{50} (µg/mL)
	1 µg/mL	5µg/mL	50 µg/mL	
Berberis lycium	17.57±0.18	22.55±0.48	39.8±0.36	4.10±0.06 [b]
Solanum surattense	14.63±0.72	19.45±0.69	36.47±0.35	4.43±0.11 [a]
Ascorbic Acid	6.3±1.25	11.61±1.25	73.02±0.1	3.28±0.02 [c]

Note: Each value in the table is shown as mean ± SD (n=3), Means not sharing the same letter are significantly different at $P < 0.05$ probability level in IC_{50} column.

Table 3: Percent inhibition of PC3 cells treated with methanol extracts of *Solanum surattense* and *Berberis lycium* in MTT assay

Extract Concentration (µg/mL)	Inhibition (%)		
	Doxorubicin	*Solanum surattense*	*Berberis lycium*
7.5	38.2±3.01	8.26±3.33	1.15±1.19
15	50.4±2.15	10.52±4.05	1.2±1.88
30	80.8±0.23	35.93±1.29	4.61±3.81
IC_{50}	14.13±2.08[a]	42.01±1.31[b]	309.06±3.55[c]
Regression equation	y = 1.9124x + 23	y = 1.2961x - 4.445	y = 0.1643x - 0.555
R^2	R^2 = 0.9973	R^2 = 0.934	R^2 = 0.9005

Note: Each value in the table is shown as mean ± SD (n=3), Mean values not sharing the same superscript letters (a, b, c) in IC_{50} row are significantly different at $P < 0.05$.

4. DISCUSSION

Plants as natural sources of bioactive compounds serve as a promising avenue for various ailments including cancer, diabetes and cardiovascular diseases [11][12][13]. In the present study *B. lycium* and *S. surattense* were evaluated for bioactive compounds and biological activities including antioxidant and anticancer. *B. lycium* was found to contain high concentrations of phenolic and flavonoid contents. Mughal TA and coauthors [14] also reported presence of high concentrations of phytochemicals such as saponins, tannins, phenols, and flavonoids in the methanolic extracts of *B. lycium.* A similar study also reported the correlation between bioactive compounds and biological activities in *Piper betle* extracts [15].

The antioxidant potential of *B. lycium* and *S. surattense* was assessed using the DPPH radical scavenging assay. In the DPPH assay, the decolorization of purple colored free DPPH radical, upon reduction by antioxidant compounds, is measured spectrophotometrically at 517 nm. The methanol extracts of both plants exhibited antioxidant potential against the DPPH radical at all tested concentrations that established a concentration-dependent response with ascorbic acid serving as the positive control. The *B. lycium* extract exhibited stronger free radical scavenging activity compared to the *S. surattense* extract, suggesting a richer profile of antioxidant compounds in *B. lycium*. A similar study also reported the presence of antioxidant phytochemicals in extracts of *B. lycium* roots [16]. Other studies have also reported that plants with higher phenolic and flavonoid contents tend to exhibit greater antioxidant activity [17]. *B. lycium* contains compounds such as berberine, palmatine, and jatrorrhizine which contribute to its antioxidant potential. Moreover, *B. lycium* possesses a range of polyphenols, which include quercetin, chlorogenic acid, syringic acid, coumaric acid, gallic acid, bergenin, and caffeic acid. These polyphenols are well known for their antiradical activities and possess various health improving properties [18].

The results for *S. surattense* were consistent with previous studies, suggesting its antioxidant potential [19][20]. A previous study also reported the antioxidant activity of *S. surattense* from India, with IC_{50} of 10.15 µg/mL, which is higher than the 4.43 µg/mL calculated in the present study [21]. This suggests the effect abiotic factors of environment, like soil, temperature and geography on phytochemical content and biological activities [22]. Previously, Anwar J and coauthors also reported the variations in phytochemical content and antioxidant activity of *Murraya koenigii* [23].

The extraction of important metabolites is directly correlated with the type of method used and nature of the solvent. In the current study, the maceration method was used along with methanol as an extraction solvent. Similarly, previous studies have used methanol or hydroalcoholic solvents for extraction [24]. Methanol is highly preferred for the extraction of phytochemicals due to its ability to extract diverse range of compounds, including both polar and non-polar phytochemicals. Other factors that affect active metabolite productions include the type and part of the plant used, harvesting season, geographical location, and soil composition. Seasonal variations affect the antibacterial potential of *B. lycium*, with winter and early spring harvests exhibiting the strongest activity [25].

Our study investigated the anticancer activity of methanol extracts from *B. lycium* and *S. surattense* against PC3 cells using the MTT assay. As expected, the assay measured the metabolic activity of viable cells through the conversion of MTT into formazan crystals. *S. surattense* exhibited greater anticancer activity

than *B. lycium,* suggesting that it may contain phytochemicals other than phenolics and flavonoids that contribute to its cytotoxic effects. This is consistent with previous studies, which have reported that *S. surattense* contains steroid glycosides that exhibit significant anticancer potential against the NIH-3T3 fibroblast cancer cells, with an IC_{50} of 7.55±1.5 µg/mL [26]. Furthermore, plants containing phytochemicals such as alkaloids, terpenes, and saponins, can exhibit anticancer activity [27][28].

Previously, a strong antiproliferative activity of water/ethanolic extract of *B. lycium* Royle against the HepG2 cancer cell line was reported [29]. The observed low cytotoxicity of *B. lycium* in the present study warrants further investigation to reconcile the discrepancies with previous reports. Several factors might contribute to these differences. Variations in plant source (including geographic origin, growth conditions) and extraction methods can significantly influence the concentration and composition of bioactive compounds within the extracts. Such differences may explain the lack of observed activity in our extracts compared to those used in previous studies. Additionally, cancer cells exhibit diverse biological properties, making them susceptible to certain compounds than others.

5. CONCLUSION

This study investigated the potential health benefits of *B. lycium* and *S. surattense* by analyzing their phytochemicals and biological activities which include antioxidant and anticancer. Both extracts contained potential bioactive compounds, as indicated by their total phenolic and flavonoid content. *B. lycium* exhibited a particularly strong antioxidant profile, suggesting its potential as a natural antioxidant source. The evaluation of anticancer activity against human prostate cancer cells suggests that *S. surattense* is a potential source of antioxidant/bioactive compounds. Future studies should investigate/explore the influence of extraction methods, plant source variations, and concentration ranges to fully understand the therapeutic potential of these plants.

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