Journal of Applied Pharmaceutical Research
Volume 13 Issue 3, Year of Publication 2025, Page 247 – 258
DOI: https://doi.org/10.69857/joapr.v13i3.1215

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | JOAPR
www.japtronline.com *ISSN: 2348 – 0335*

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT POTENTIAL, AND CYTOTOXIC ACTIVITY OF EXTRACTS OF QUISQUALIS INDICA L.

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Article Information
Received: 2nd May 2025
Revised: 10th June 2025
Accepted: 21st June 2025
Published: 30th June 2025

Keywords
Quisqualis indica, flavonoids, DPPH, MTT, antioxidant, cytotoxicity.

ABSTRACT
Background: The present study investigates the antioxidant and cytotoxic potential of the 50% hydroalcoholic extract of *Quisqualis indica* leaves. **Methodology:** Phytochemical screening was conducted to determine the presence of phenolics and flavonoids. TPC and TFC were analyzed using the Folin–Ciocalteu method and the aluminum chloride colorimetric assay, respectively. The antioxidant activity was evaluated through the DPPH radical scavenging assay at concentrations ranging from 10 to 100 µg/mL. Cytotoxicity was assessed in A549 human lung carcinoma cells using the MTT assay with extract concentrations from 0 to 1000 µg/mL. **Results and Discussion:** Phytochemical analysis confirmed the presence of phenolics and flavonoids, with total phenolic content measured as 9.25 ± 0.081 mg gallic acid equivalents (GAE)/g 50% hydroalcoholic extract and total flavonoid content as 4.33 ± 0.24 mg quercetin equivalents (QE)/g 50% hydroalcoholic extract. Antioxidant activity was assessed using the DPPH radical scavenging assay across extract concentrations ranging from 10 to 100 µg/mL. The 50% hydroalcoholic extract exhibited a dose-dependent antioxidant effect with an IC_{50} value of 48.56 µg/mL. Cytotoxicity was evaluated against A549 human lung carcinoma cells using the MTT assay, with treatments administered at concentrations ranging from 0 to 1000 µg/mL. The extract demonstrated significant cytotoxicity with an IC_{50} value of 4.76 µg/mL. **Conclusion:** These findings suggest that *Q. indica* may serve as a potential source of bioactive compounds with antioxidant and anticancer activities, warranting further investigation through *in vivo* and mechanistic studies.

INTRODUCTION

Quisqualis indica (*Q. indica*), commonly known as Chinese honeysuckle, quisqualis, Rangoon creeper, and red jasmine, is a vine native to tropical Asia that bears red flowers. It is a climbing shrub with oblong-lanceolate elliptic leaves and fragrant flowers which bloom on terminal drooping branches [1][2]. It contains essential components such as rutin, quisqualic acid, trigonelline, L-proline, and L-asparagine. In addition, the plant contains various secondary metabolites, including tannins, flavonoids, steroids, hydrocarbons, amino acids, sugars, and phenols [3][4][5]. Modern pharmacological research supports the traditional claims of *Q. indica*, showing that it possesses anti-inflammatory, anti-diarrheal, and antirheumatic properties. Recent research has found that the plant exhibits antibacterial,

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anthelmintic, hypoglycemic, antimicrobial, fungicidal, antioxidant, anticancer, and immunomodulating properties [6][7][8]. Combining traditional uses and plant properties *Q. indica* is a promising candidate for further research and possible use in herbal medicine [9]. One of the most notable compounds discovered through chemical analysis is Quinoline-4-CarboNitrile (QCN), a volatile alkaloid isolated from the flower extracts of plants. In addition, other essential compounds found in *Q. indica* include linalool oxide, linalool benzoate, and benzylbenzoate [10][11][12]. These chemicals play a crucial role in determining the properties of the plant and have been extensively studied for their biological activity [13]**.** Its complex chemical structure and traditional medicinal uses make it an attractive subject for further investigation in the field of pharmacology and drug development. Researchers are keen to explore the full therapeutic potential of this plant and to understand the mechanisms behind its beneficial effects [14]**.** It is a tropical flowering ligneous vine or vigorous climber up to 2.5 m to 8 m in length, found in the Philippines, India, and Malaysia [15][16]. The plant-chemical analysis of *Q. indica* has been extensively investigated in various studies. Analytical High Performance Thin-Layer Chromatography-Mass Spectroscopy (HPTLC-MS) was used to examine the chemical composition of the plant [17][18]**.** Examination of the methanol extract from the leaves of *Q. indica* revealed significant compounds such as rutin, quisqualis acid, trigonelline, L-proline, and L-asparagine [19]. Plant extracts reduce Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), and Congestive Heart Failure (CHF), while increasing High Density Lipoprotein (HDL). These effects are thought to be related to the inhibition of lipid peroxidation and the presence of antioxidants in the plant. In addition, cytotoxicity of *Q. indica* was also of interest in tumour studies, as preliminary findings indicate that some components of this plant may induce apoptosis in tumour cells. While these findings are promising, more in-depth studies are needed to determine both the clinical relevance and the underlying biochemical processes [20][21].

MATERIAL AND METHODS

Plant material

Fresh leaves of *Q. indica* have been harvested from the local Varanasi area of Uttar Pradesh, India. For identification and taxonomic verification, a sample of the plant was sent to Banaras Hindu University in Varanasi, India, which confirmed that the plant sample was genuine (voucher specimen no. Combret 2022/1). Samples were shade-dried for two weeks. After drying, the plant material was ground well with an electric mill to a fine powder and stored in well-marked airtight containers.

Preparation of the Extract of *Quisqualis indica* Leaves

In this study, the Soxhlet extraction method was used to extract bioactive compounds from the leaves of *Q. indica*. The subsequent extraction process was carried out with solvents of increasing polarity, namely petroleum ether, hydroalcohol (50%), and aqueous. The extraction process was in order of priority, starting with petroleum ether, then 50% hydroalcohol, and finally aqueous.

Extraction Procedure

In total, 100 g of *Q. indica* leaf powder was placed in the amber liquid crystal flask, and 250 mL of the appropriate solvent was added. The extraction of the plant material was carried out in a Soxhlet apparatus at a temperature of 600°C for eight hours with constant stirring to achieve maximum extraction efficiency. After the extraction process, the mixture was centrifuged at 1500 rpm for 15 minutes. The supernatant was collected and re-extracted with the same solvent to ensure a thorough extraction of the plant material. This sequential extraction process was repeated for each solvent in the same order. After the extraction was completed, the solvent from the extracted material was evaporated under vacuum to obtain a dry extract suitable for further phytochemical & biochemical analysis [22].

Collection of leaves
↓
Washing and shade drying
↓
Powdering of dried leaves
↓
50% ethanol used as a solvent
↓
Soxhlet extraction (6 hours)
↓
Concentration using a rotary evaporator
↓
Storage of extract at 4°C

Preparation of Stock Solution

For the formulation of the stock solution, a 2 mg/mL solution, 100 mg of petroleum ether, 50 % alcohol, and aqueous extracts were accurately measured and dissolved in the respective 50 mL solvents. The prepared solutions were stored in a refrigerator for further analysis.

Preliminary Phytochemical Screening

Preliminary phytochemical examination of *Q. indica* leaves was carried out using standard protocols. Petroleum ether, alcohol (50%), and aqueous extracts were used for testing. The following tests were used to identify the presence of different phytoconstituents: carbohydrates (Molisch, Benedict's, and Fehling's tests), alkaloids (Mayer's, Wagner's, Dragendorff's, and Hager's tests), saponin glycosides (Foam and Haemolysis tests), anthraquinone glycosides (Borntrager's and Modified Borntrager's tests), flavonoids (Shinoda and Ferric chloride tests), cardiac glycosides (Legal, Baljet, and Keller-Kiliani tests), steroids (Liebermann-Burchard test), phytosterols (Salkowski's test), proteins and amino acids (Xanthoproteic, Ninhydrin, and Millon's tests), and tannins (Ferric chloride and Gelatin tests). Tests revealed the presence of alkaloids, anthraquinone glycosides, flavonoids, tannins, phenolic compounds, sugars, sucrose, saponins, phytosterols, and triterpenes in plant extracts [23][24].

Total Polyphenol Content (TPC)

The Folin-Ciocalteau test was used to measure the total polyphenol content of the test mixture. For this test, 0.5 mL of each extract was mixed in the Eppendorf tube with 2.5 mL of 0.2 N Folin-Ciocalteau reagent. After 5 minutes, 2 mL of 75 g/L of sodium carbonate (5% w/v) was added to the mixture and incubated at 400 °C for 120 minutes. The absorbance of the solution was subsequently measured at a wavelength of 760 nm. The TPC was calculated as:

$$\boldsymbol{TPC}\ (\boldsymbol{mg}\frac{\boldsymbol{GAE}}{\boldsymbol{g}}\boldsymbol{extract}) = \frac{\boldsymbol{C-V}}{\boldsymbol{M}},$$

Where:

C = Conc. of gallic acid from the standard curve (mg/mL),

V = volume of extract (mL), **M** = mass of extract used (g)

Total Flavonoid Content (TFC)

The total flavonoid content was determined by the Folin-Ciocalteu assay using a calibration curve based on quercetin. The procedure for measuring total flavonoid yield was based on a previously published method. This involved adding 100 µL of the extract sample to 860 µL of a 35% (v/v) mixture of water and methanol, and 40 µL of a 5% (w/v) AlCl3 solution in 0.5 M CH_3COONa solution. Absorbance was measured at 415 nm after incubation. Total flavonoid content was expressed as milligrams of quercetin equivalent per gram (mg QE/g) of dry plant material. Accurate quantification was achieved using a calibration curve prepared with quercetin. The TFC was calculated using the equation [25][26].

$$\boldsymbol{TFC}\ (\boldsymbol{mg}\frac{\boldsymbol{QE}}{\boldsymbol{g}}\boldsymbol{extract}) = \frac{\boldsymbol{C-V}}{\boldsymbol{M}},$$

Where:

C=concentration of quercetin from the standard curve (mg/mL), **V**=volume of extract (mL), **M**=mass of extract used (g)

ANTI-OXIDANT ACTIVITY

Free Radical (DPPH) Scavenging Activity

The petroleum ether, 50% hydroalcohol, and aqueous extracts of *Q. indica* leaves were assessed for their free radical scavenging activity using the stable radical compound 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH). The ability of different compounds in the extract to donate hydrogen atoms or electrons was evaluated by measuring the discoloration rate or reduction in absorbance of the purple DPPH solution in methanol. For the assay, 2 mL of each extract concentration was added to 2 mL of 0.004% DPPH solution in methanol. After 90 minutes of storage in the dark at room temperature, the absorbance of the sample at 517 nm was read against the control. The free radical (DPPH) scavenging activity was calculated using the following equation[27][28]:

$$\boldsymbol{DPPH\ scavening}\ (\%) = \frac{\boldsymbol{A_0 - A_1}}{\boldsymbol{A_0}} \times \boldsymbol{100},$$

Where:

A_0 = absorbance of control (without extract)

A_1 = absorbance in the presence of extract.

Superoxide Radical Scavenging (ROS) activity

For the calculation of the superoxide scavenging activity, 100 µL of *Q. indica* extract and standard curcumin were diluted in three test tubes. Then add 3 ml of a reaction mixture prepared with 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EthyleneDiamine Tetra Acetic acid (EDTA) and 0.1 mg Nitro-Blue Tetrazolium chloride (NBT). The reaction was initiated by exposing the mixture to light for 90 seconds. A negative control was set in which the reaction mixture was illuminated without introducing a plant extract or standard, and a blank was set in which the reaction mixture was illuminated without an extract or standard. After illumination, the absorbance of each reaction mixture was measured by a spectrophotometer at 590 nm, and the percentage of superoxide radicals scavenged was calculated by measuring the reduction in absorbance compared with the control by the formula [28][29][30]:

$$Percentage\ scavening = \frac{A_0 - A_1}{A_0} \times 100,$$

Where:

A_0 = absorbance of control (without extract)

A_1 = absorbance in the presence of extract.

IN-VITRO CYTOTOXICITY EVALUATION

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay

The cytotoxicity of the samples provided was determined using a standardized test in the A549 (NCCS Pune) cell line. Cells (10000 cells per well) were cultured for 24 hours on 96 well plates in DMEM (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO_2. The following day, the samples were tested at various concentrations: 0, 1, 10, 50, 100, 250, 500, and 1000 µg/mL. After incubation for 24 hours, the MTT solution (0, 1, 10, 100, 250, 500, 1000 µg/mL) was added to the culture medium and further incubated for 2 hours. At the end of the experiment, the culture supernatant was separated, and the matrix of the cell layer was dissolved in 100 µL DiMethylSulfOxide (DMSO–SRL- Cat no.- 67685) and read at 540 nm and 660 nm by the ELISA plate reader (IMark, Biorad, USA). The IC_{50} values (the concentration at which 50% of cells are inhibited) were calculated using GraphPad Prism 6 and expressed in micrograms per milliliter (µg/mL). The images were taken with a camera (Olympus EK2) and an AmScope digital camera 10 MP Aptima CMOS [31][32][33].

Statistical analysis

The Analysis of Variance (ANOVA) was performed using a general treatment structure (randomized blocks) for both the total phenolic and total flavonoid analyses. The data were analysed in GenStat 7 (version 7.2.0.220). The results of these experiments were presented in graphic form. Student t-tests and linear regression analysis (using SPSS version 16.0, 2007) were employed, along with Excel graphing, to investigate the relationship between variables.

RESULT AND DISCUSSION

Extractive yield of *Quisqualis indica* leaves

The extraction yield was determined for various solvents, including petroleum ether, 50% alcohol, and aqueous, by the Soxhlet extraction method. The percentage yield for each extract was calculated using the following formula:

$$Percentage\ yield\ of\ extract = \frac{Final\ weight\ of\ extract}{Initial\ weight\ of\ plant\ material} \times 100.$$

The extractive yield values obtained for *Q. indica* leaf samples are summarised in Table 1 & illustrated graphically in Figure 1.

Table 1: The extractive yield of *Q. indica* leaf extract was evaluated in petroleum ether, 50% hydroalcohol, and aqueous solvents

S.No.	Solvent	Percentage Yield
01	Petroleum ether	35
02	50 % Hydroalcohol	18
03	Aqueous	4.5

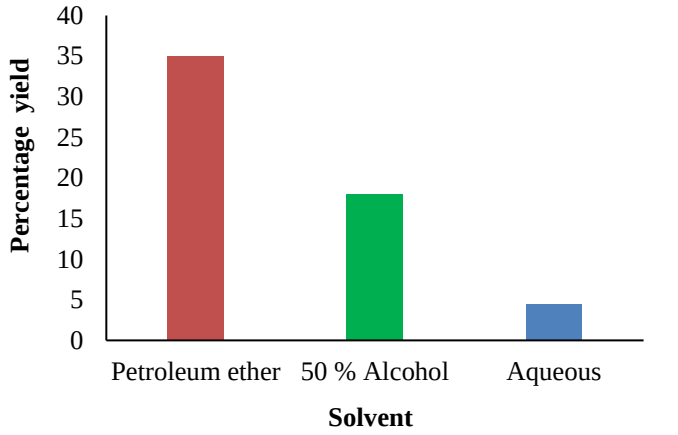

Figure 1: The extractive yield of *Q. indica* is represented in percentage: the orange bar indicates that petroleum ether produced the highest extractive yield at 35%, the green bar shows a 50% hydroalcohol yield of 18%, and the blue bar represents the aqueous yield of 4.5%.

Results show that petroleum ether yielded the highest extractive percentage (35%), followed by 50% hydroalcohol (18%) and aqueous extract (4.5%). This indicates that non-polar compounds are more efficiently extracted from petroleum ether than from polar solvents, such as alcohols and aqueous solutions.

QUALITATIVE PHYTOCHEMICAL SCREENING

Preliminary Phytochemical Screening Results

Qualitative phytochemical analysis of petroleum ether, 50% alcohol & aqueous extract revealed the presence of several phytochemical compounds. The results are summarised in Table 2.

Table 2: Preliminary phytochemical screening of *Q. indica* revealed the presence of various phytochemicals in the leaf extracts obtained using petroleum ether, 50% hydroalcohol, and aqueous.

Phytochemical Analyzed	Test Performed	Results		
		Petroleum ether extract	50% Hydroalcoholic Extract	Aqueous Extract
Carbohydrates	Molish test	-	-	+
	Benedict's test	-	-	+
	Fehling's test	-	-	+
Alkaloids	Mayer's test	+	-	-
	Wagner's test	+	-	-
	Dragendroff's test	-	-	-
	Hager's test	-	+	-
Saponin Glycosides	Foam test	+	+	+
	Haemolysis test	-	-	+
Anthraquinone Glycosides	Borntrager's	+	+	+
	Modified Borntrager's	-	+	+
Flavonoids	Shinoda test	+	+	+
	Ferric chloride	-	+	+
Cardiac Glycosides	Legal test	-	+	+
	Baljet test	-	+	+
	kellerkiliani test	-	+	+
Phytosterols	Salkowski's	+	-	-
Proteins and Amino Acids	Xanthoproteic	-	-	-
	Ninhydrin	-	-	-
	Millon's	-	+	+
Tannins	Ferric chloride	-	+	+
	Gelatin test	-	+	+

Phytochemical examination of the extracts of *Q. indica* leaf extract revealed that the carbohydrates were present only in the aqueous extract. Alkaloids were present only in the petroleum ether extract, whereas glycosides of saponins and anthraquinones were present in all extracts.

Flavonoids and cardiac glycosides have been reported in hydroalcoholic and aqueous extracts. The phytosterols were only found in the extract of petroleum ethers. Protein and amino acid content were absent globally, except for a positive Millon test for hydroalcoholic and aqueous extracts. The tannins were present in the 50% hydroalcoholic and aqueous extracts.

Estimation of total phenolic content and Total flavonoid Content: The quantities of phenol and flavonoids in the different solvents are shown in Figures 2, 3, 4, and in Table 3.

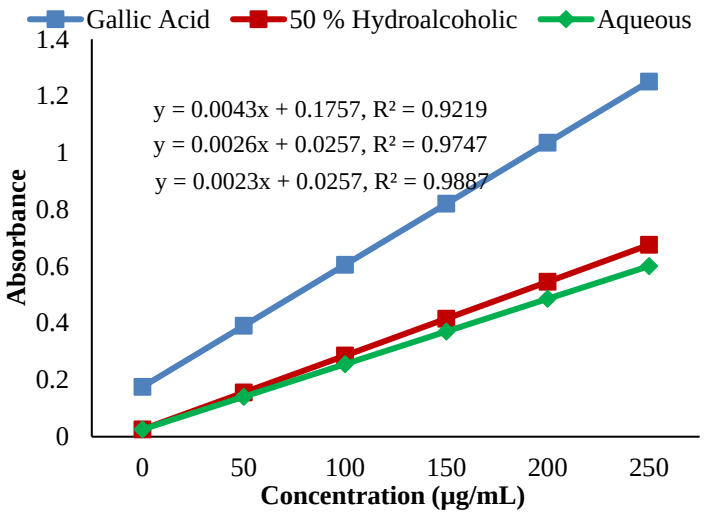

Figure 2: Absorbance at 760 nm for various concentrations (µg/ml) shows TPC of gallic acid (blue), 50% hydroalcoholic extract (red), and aqueous extract (green) of *Q. indica*.

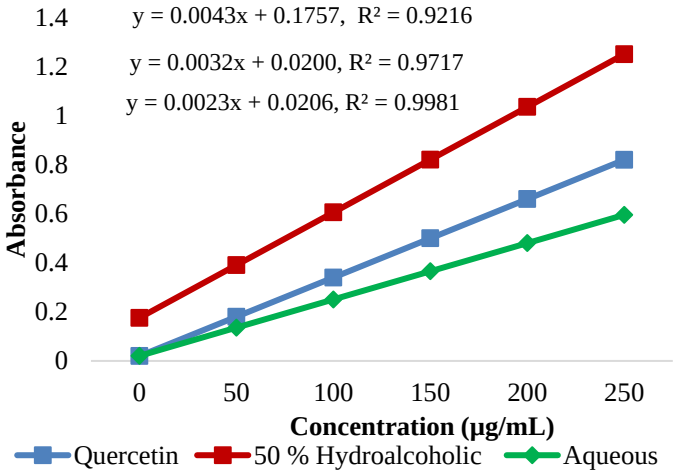

Figure 3: Absorbance at 415 nm for various concentrations (µg/ml) shows TFC of quercetin (blue), 50% hydroalcoholic extract (red), and aqueous extract (green) of *Q. indica*.

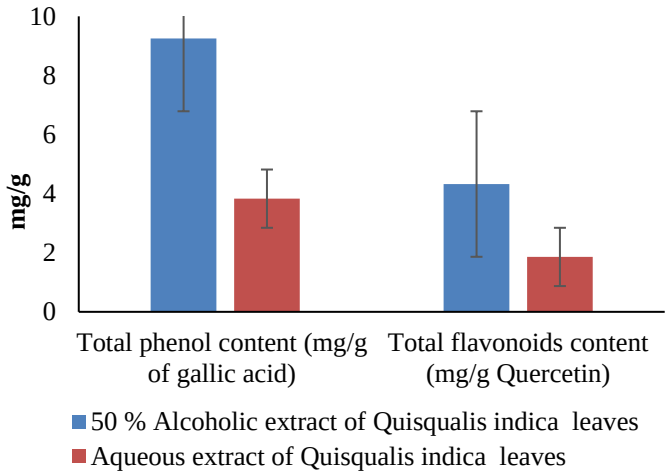

Figure 4: Total phenolic and flavonoid content of *Q. indica*: the blue bar represents the 50%hydroalcoholic extract, while the orange bar represents the aqueous extract.

Table 3: Total phenolic and flavonoid content of *Q. indica* extracts using 50% hydroalcohol and aqueous solvents.

SN	Extracts	Total Phenolic Content *(mg/g gallic acid)**	Total Flavonoid Content *(mg/g quercetin)**
1	50% Hydroalcoholic extract of *Q. indica* leaves	9.25±0.081	4.33±0.240
2	Aqueous extract of *Q. indica* leaves	3.84±0.091	1.86±0.620

**All estimates are communicated as mean ± SEM for three measurements*

The total phenolic and flavonoid contents in the extracts of *Quisqualis indica* were quantified as gallic acid and quercetin equivalents, respectively. The 50% hydroalcoholic extract of *Q. indica* leaves showed a total phenolic content of 9.25 ± 0.081 mg/g (gallic acid equivalents) and a total flavonoid content of 4.33 ± 0.24 mg/g (quercetin equivalents). In contrast, the aqueous extract contained 3.84 ± 0.091 mg/g of phenolic compounds and 1.86 ± 0.62 mg/g of flavonoids. These results indicate that the 50% hydroalcoholic solvent is more efficient in extracting phenolic and flavonoid compounds than the aqueous solvent.

ANTIOXIDANT ACTIVITY

Free Radical (DPPH) Scavenging Activity

The scavenging activity of *Q. indica* extracts at varying concentrations was assessed and compared to that of ascorbic acid, a well-established natural antioxidant. The percentage inhibition is illustrated in Figure 5 and summarized in Table 4. *Q. indica* leaf extracts were evaluated for antioxidant activity at concentrations ranging from 10 to 100 µg/mL, with ascorbic acid used as a reference standard at 50 µg/mL. All extracts showed a concentration-dependent increase in percentage inhibition of DPPH radicals.

At 10 µg/mL, the percentage inhibition was 15.52% for the petroleum ether extract, 30.35% for the 50% hydroalcoholic extract, and 22.48% for the aqueous extract. At 25 µg/mL, inhibition increased to 30.85%, 46.25%, and 35.16%, respectively. At 50 µg/mL, the hydroalcoholic extract exhibited 50.23% inhibition, followed by the aqueous extract at 43.56% and the petroleum ether extract at 40.21%.

At 75 µg/mL, inhibition further increased to 45.36% (petroleum ether), 54.67% (hydroalcoholic), and 48.35% (aqueous). At the highest concentration tested (100 µg/mL), the hydroalcoholic extract again demonstrated the highest antioxidant activity (61.25%), followed by the aqueous (55.98%) and petroleum ether (51.47%) extracts. In comparison, ascorbic acid exhibited a consistent 40% inhibition at 50 µg/mL.

The IC_{50} values were calculated as 48.56 µg/mL for the 50% hydroalcoholic extract, 80.41 µg/mL for the aqueous extract,

and 93.99 µg/mL for the petroleum ether extract. These results indicate that the 50% hydroalcoholic extract possesses the highest antioxidant potential among the extracts, followed by the aqueous and petroleum ether extracts.

Table 4: Absorbance values of different extracts of *Q. indica*: petroleum ether, 50% hydroalcoholic, and aqueous extracts.

Conc.(µg/mL)	Petroleum Ether Extract %	50% Hydroalcoholic Extract %	Aqueous Extract %	Ascorbic Acid (Std) %
10	15.52	30.35	22.48	–
25	30.85	46.25	35.16	–
50	40.21	50.23	43.56	**40.00**
75	45.36	54.67	48.35	–
100	51.47	61.25	55.98	–
IC_{50} (µg/mL)	**93.99**	**48.56**	**80.41**	–

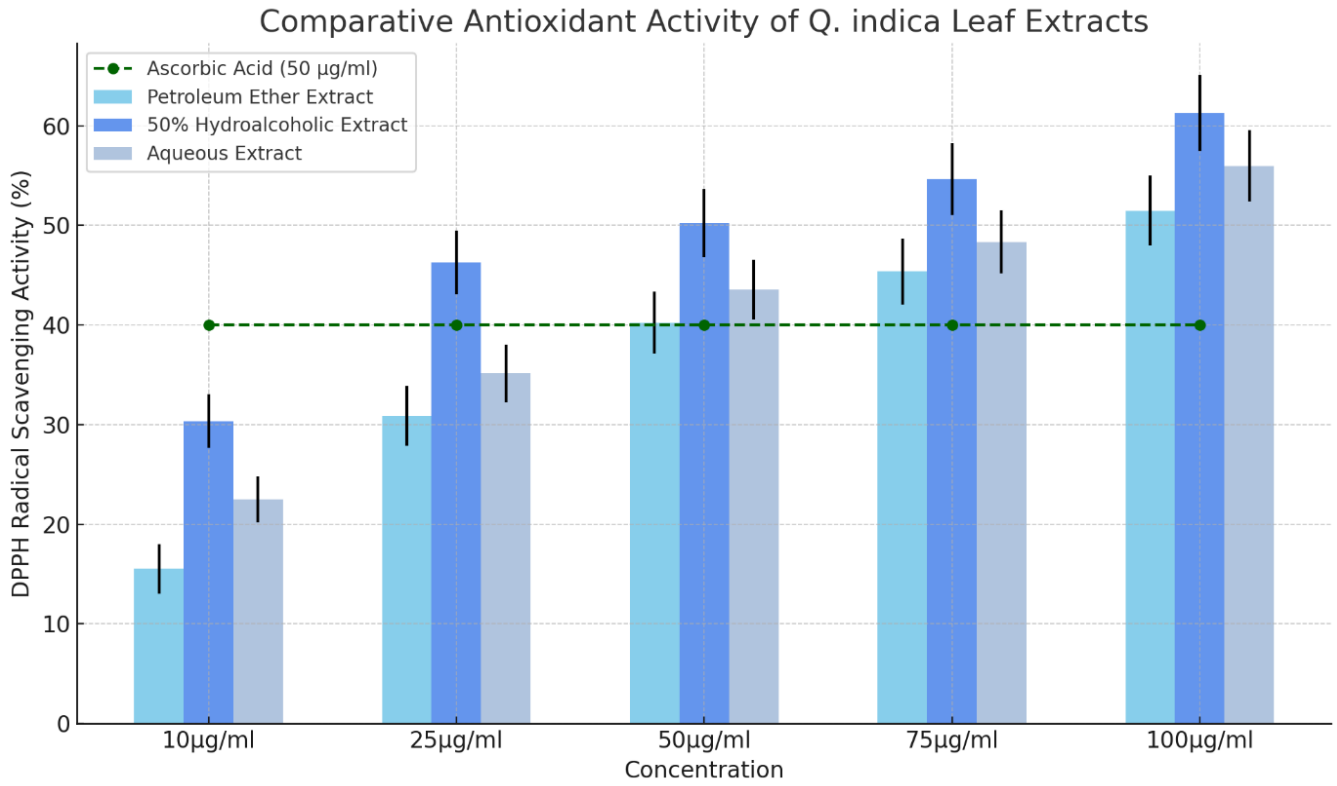

Figure 5: DPPH radical scavenging activity (%) of *Petroleum Ether*, *50% Hydroalcoholic*, and *Aqueous extracts* of *Q. indica* leaves, along with ascorbic acid (50 µg/mL) as the standard.

Superoxide Radical Scavenging (ROS) of *Q. indica*

The antioxidant activity of Q. indica leaf extracts was evaluated at different concentrations (10–100 µg/mL) & compared with curcumin, a recognized anti-inflammatory agent (Table 5 & Figure 6).

Table 5: Superoxide Radical Scavenging Assay of *Q. indica* extracts (petroleum ether, 50% hydroalcoholic, and aqueous) at various concentrations.

Conc. (µg/mL)	Petroleum Ether Extract (%)	50% Hydroalcoholic Extract (%)	Aqueous Extract (%)	Curcumin (%)
10	2.00	8.00	9.00	35.00
25	4.85	16.00	18.00	60.00
50	11.21	35.00	21.53	72.00
75	18.36	51.00	38.00	85.00
100	25.47	65.00	49.00	90.00

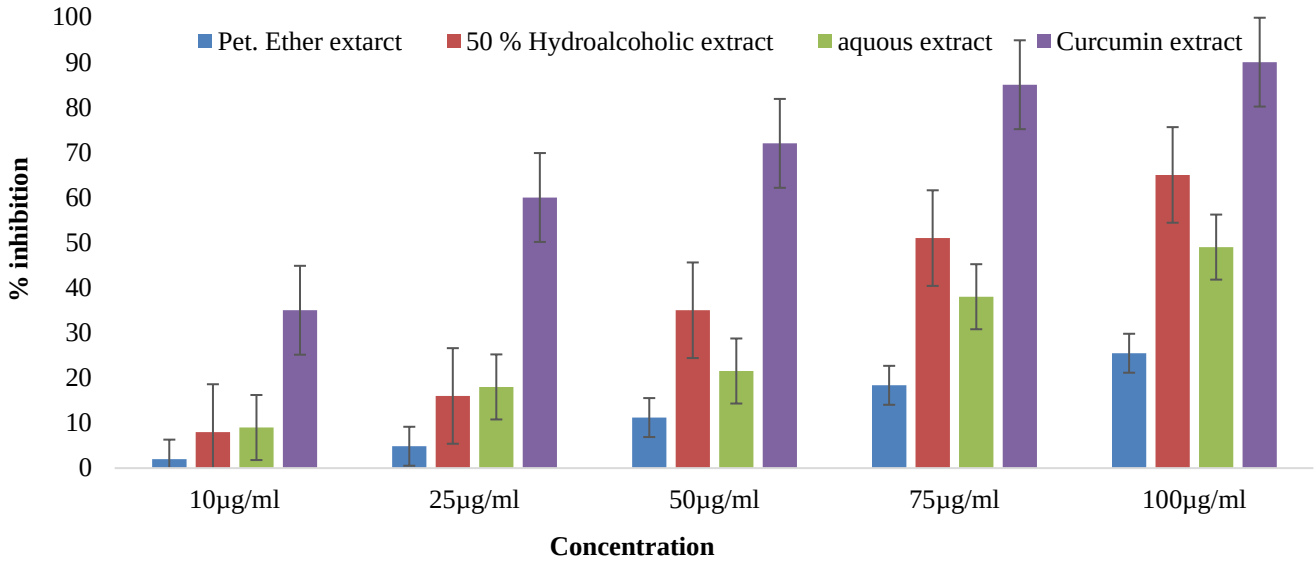

Figure 6: Superoxide Radical Scavenging activity of *Q. indica* extracts at various concentrations. Blue bars indicate petroleum ether extract, orange bars indicate 50% hydroalcoholic extract, gray bars indicate aqueous extract, and yellow bars indicate curcumin (standard).

The superoxide radical scavenging activity of *Quisqualis indica* leaf extracts was assessed at concentrations ranging from 10 to 100 µg/mL, using curcumin as a reference antioxidant. At 10 µg/mL, the percentage inhibition was 2.00% for the petroleum ether extract, 8.00% for the 50% hydroalcoholic extract, and 9.00% for the aqueous extract, while curcumin showed 35.00% inhibition. At 25 µg/mL, inhibition increased to 4.85% (petroleum ether), 16.00% (hydroalcoholic), and 18.00% (aqueous), with curcumin showing 60.00% inhibition. At 50 µg/mL, the hydroalcoholic extract exhibited the highest inhibition among the extracts (35.00%), followed by the aqueous extract (21.53%) and the petroleum ether extract (11.21%). Curcumin exhibited 72.30% inhibition.

A similar trend was observed at 75 µg/mL, with inhibition values of 51.00% (hydroalcoholic), 38.00% (aqueous), and 18.36% (petroleum ether); curcumin was inhibited by 85.00%. At the maximum tested concentration of 100 µg/mL, the 50% hydroalcoholic extract achieved 65% inhibition, followed by the aqueous extract (49%) & the petroleum ether extract (25.47%), while curcumin demonstrated the highest inhibition at 90%. These results indicate that the 50% hydroalcoholic extract of *Q. indica* exhibits the most potent superoxide radical scavenging activity among the tested extracts in a concentration-dependent manner. However, its efficacy remains lower than that of curcumin.

In-vitro anticancer activity by MTT assay *of Q. indica*

MTT Assay- A549

Cytotoxicity of samples submitted in the A549 cell line (NCCS Pune) was determined by MTT. Cytotoxic effects in the test sample were determined at a range of concentrations from 0 to 1000 µg/mL, with dose-dependent reduction in cell viability (Table 6 and Figure 7).

MTT Assay for Cytotoxicity Evaluation

The cytotoxic activity of the 50% hydroalcoholic extract of *Q. indica* was evaluated using the MTT assay on A549 human lung carcinoma cells. Cells were exposed to varying concentrations of the extract (0–1000 µg/mL) for 24 hours. Cell viability decreased in a dose-dependent manner, indicating potential anticancer activity of the extract. The IC_{50} value—the concentration at which 50% of cell viability was inhibited—was calculated using Prism-6 software and found to be 4.76 µg/mL. These results suggest that the hydroalcoholic extract of *Q. indica* exhibits moderate cytotoxicity against A549 cells, warranting further exploration for its potential anticancer properties. Doxorubicin (1–10 µg/mL) was used as a positive control due to its well-established cytotoxic effect on lung cancer cell lines. A vehicle-treated group served as the negative control. Cell viability was calculated as a percentage of the control group. IC_{50} values were determined from dose–response curves plotted using nonlinear regression in GraphPad Prism.

Table 6: Superoxide Radical Scavenging Assay results of *Q. indica* extract at various concentrations (µg/mL). The table shows the sample concentration, mean percentage inhibition, standard deviation (SD) & standard error of the mean (SEM).

Sample Conc. (µg/mL)	Mean Cell Viability (%)	Standard Deviation (SD)	Standard Error of Mean (SEM)
0	100.00	19.05	9.52
1	51.91	7.26	3.63
10	36.94	13.05	6.52
50	29.28	8.70	4.35
100	28.49	6.01	3.01
250	26.91	3.34	1.67
500	21.62	13.82	6.91
1000	12.95	6.04	3.02

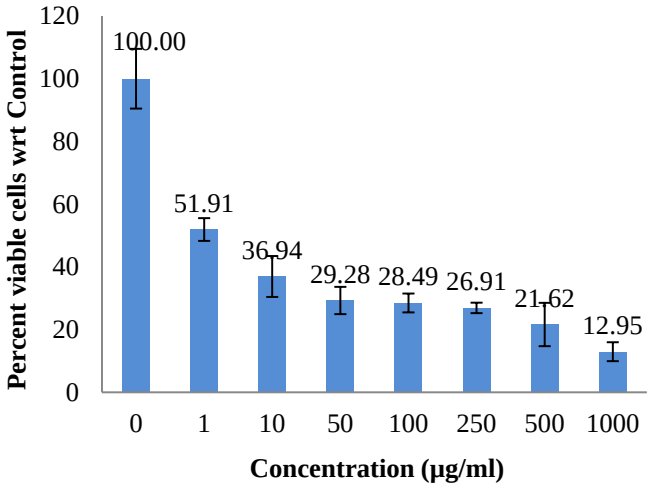

Figure 7: MTT assay of A549 cells treated with *Q. indica* extract. The graph shows a dose-dependent decrease in A549 cell viability, with viability reducing from 100% (control) to 12.95% at 1000 µg/mL, indicating the cytotoxic potential of the extract.

The cytotoxic activity of the 50% hydroalcoholic extract of *Q. indica* was assessed using the MTT assay on A549 human lung carcinoma cells. Cells were treated with varying concentrations of the extract (0–1000 µg/mL) for 24 hours. At the control concentration (0 µg/mL), cell viability was 100% (SD: 19.05, SEM: 9.52). Viability decreased in a dose-dependent manner: at 1 µg/mL, viability was slightly reduced (mean: 92%, SD: 7.26, SEM: 3.63); at 10 µg/mL, viability dropped to 36.94% (SD: 13.05, SEM: 6.52); and at 50 µg/mL, it further declined to 29.28% (SD: 8.70, SEM: 4.35).

Higher concentrations showed continued cytotoxic effects: 28.49% at 100 µg/mL (SD: 6.01, SEM: 3.01), 26.91% at 250 µg/mL (SD: 3.34, SEM: 1.67), 21.62% at 500 µg/mL (SD: 13.82, SEM: 6.91), and a minimum viability of 12.95% at 1000 µg/mL (SD: 6.04, SEM: 3.02). The IC_{50} value—the concentration at which 50% of cell viability is inhibited—was calculated to be approximately 4.76 µg/mL, indicating that the extract exhibits significant cytotoxic potential. In comparison, the standard chemotherapeutic agent exhibited an IC_{50} of 0.58 µg/mL under identical conditions, validating the assay's sensitivity. These results indicate that the extract possesses moderate cytotoxic potential relative to the standard chemotherapeutic agent. These results suggest that the 50% hydroalcoholic extract of *Q. indica* possesses notable concentration-dependent cytotoxicity, although it is less potent than the established anticancer drug.

DISCUSSION

This study comprehensively evaluates the phytochemical profile, antioxidant potential, and cytotoxic properties of *Q. indica* extracts prepared using solvents of varying polarity. Among all extracts, the 50% hydroalcoholic extract consistently demonstrated superior bioactivity, aligning with its higher content of total phenolics (9.25 ± 0.081 mg/g GAE) and flavonoids (4.33 ± 0.24 mg/g QE). These bioactive constituents are known to play critical roles in antioxidant defense and anticancer mechanisms, thus validating the effectiveness of this extraction method.

Solvent polarity was observed to influence both extractive yield and biological activity directly. While petroleum ether yielded the highest overall extract mass (35.00%), it exhibited the lowest phenolic and flavonoid content, and correspondingly, lower antioxidant and cytotoxic activity. In contrast, the 50% hydroalcoholic extract demonstrated the highest antioxidant efficacy, with 61.25% DPPH radical inhibition at 100 µg/mL

and an IC50 of 48.56 µg/mL, indicating that mixed-polarity solvents are more effective in isolating functional bioactives, such as polyphenols and flavonoids. Similarly, in the superoxide radical scavenging assay, the hydroalcoholic extract exhibited 65.00% inhibition at 100 µg/mL, outperforming both aqueous (49.00%) and petroleum ether (25.47%) extracts.

Cytotoxic evaluation using the MTT assay against A549 lung carcinoma cells revealed a strong dose-dependent inhibition of cell viability, with the 50% hydroalcoholic extract reducing viability from 100% (control) to 12.95% at 1000 µg/mL. The calculated IC_{50} of 4.76 µg/mL indicates a potent antiproliferative effect. Importantly, when benchmarked against doxorubicin—a standard chemotherapeutic agent—the extract showed promising, though less potent, cytotoxicity. However, given its natural origin, the extract may offer advantages such as reduced systemic toxicity and side effects. The observed cytotoxic activity of the 50% hydroalcoholic extract of *Q. indica* against A549 lung cancer cells may be attributed to the presence of phenolic compounds and flavonoids, which are widely known to induce apoptosis in cancer cells through various mechanisms. Previous research has shown that such polyphenol-rich extracts may initiate mitochondrial-mediated apoptosis by upregulating pro-apoptotic proteins (e.g., Bax, caspase-3) and downregulating anti-apoptotic proteins like Bcl-2 [34][35]. Although mechanistic validation was not performed in this study, it is plausible that the extract induces cell death by disrupting the mitochondrial membrane potential, elevating reactive oxygen species (ROS), and subsequently activating intrinsic apoptotic signaling. These pathways have been documented in other medicinal plants rich in compounds such as quercetin and gallic acid—phytoconstituents previously reported in Q. indica [24][36].

Future work should aim to elucidate these cytotoxic mechanisms through targeted molecular assays, including evaluation of gene expression markers (e.g., p53, caspase-9, cytochrome c) and apoptosis quantification techniques such as flow cytometry or Annexin V/PI staining. Additionally, bioactivity-guided fractionation of the hydroalcoholic extract could help isolate and identify the specific active compounds responsible for the observed pharmacological effects. In summary, the 50% hydroalcoholic extract of *Q. indica* demonstrates the most promising therapeutic profile among all tested extracts, attributed to its richness in bioactive secondary metabolites. These results corroborate the plant's traditional medicinal uses and strongly support further pharmacological investigation, including *in vivo* validation and compound isolation. Such work may ultimately facilitate the development of novel plant-derived therapeutics for oxidative stress-related disorders and cancer.

CONCLUSION

This study highlights the pharmacological potential of Quisqualis indica, particularly its 50% hydroalcoholic leaf extract, which exhibits a superior profile of bioactive compounds, including phenols and flavonoids, key contributors to antioxidant and cytotoxic activity. Among the solvents used for extraction, the 50% hydroalcoholic system yielded the highest concentrations of total phenolics (9.25 ± 0.081 mg GAE/g) and flavonoids (4.33 ± 0.24 mg QE/g), which aligns with its strong free radical scavenging potential. The antioxidant efficacy, evaluated through the DPPH assay, revealed a dose-dependent radical neutralization for 50% hydroalcohol, with an IC50 of 48.56 µg/mL. In comparison, the reference compound, ascorbic acid, showed an IC50 of 66.67 µg/mL. Although less potent than the standard drug, the extract exhibited statistically significant activity ($p < 0.01$), which may be attributed to the synergistic effects of polyphenols and flavonoids.

Phytochemical screening confirmed the presence of alkaloids, glycosides, tannins, saponins, and phytosterols, compounds known for their medicinal properties. Additionally, the extract demonstrated moderate cytotoxicity against A549 lung cancer cells with an IC_{50} of 4.76 µg/mL, indicating potential anticancer activity. Though less cytotoxic than doxorubicin, the extract activity is promising for a crude plant extract. The presence of bioactive constituents suggests the possibility of pro-apoptotic mechanisms, warranting further investigation through the isolation of compounds and molecular studies.

In conclusion, the 50% hydroalcoholic extract of *Q. indica* exhibits notable antioxidant and cytotoxic potential, likely due to its rich polyphenolic content. While it cannot yet be classified as a therapeutic agent, it represents a promising natural source of bioactive compounds. Further pharmacological studies, including *in vivo* validation, mechanistic elucidation, and compound isolation, are recommended to explore its therapeutic potential fully.

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support and facilities provided by the Maharishi School of Pharmaceutical Sciences, Maharishi University of Information Technology, Lucknow, Uttar Pradesh, India.

AUTHOR CONTRIBUTION

The research, including study design, data collection, analysis, and manuscript drafting, was conducted by Ajay Kumar Verma. A. K. S. Rawat provided direction, oversight, and essential edits to the manuscript. The final draft of the paper was examined and approved by both authors.

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