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RESEARCH ARTICLE

Antibacterial, Antifungal, Antioxidant and Phytochemical Studies on Extracts of *Justicia beddomei* (C. B. Clarke) Bennet

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Abstract

Background: *Justicia beddomei* (C.B. Clarke) Bennet, is an important ingredient in "*Vasa*", a key medication in *Ayurveda*. There are only a few research studies of *J. beddomei.* **Objectives:** The goal of the current investigation is to perform a qualitative and quantitative screening for phytochemicals, and characterizing the antibacterial, antifungal, and antioxidant properties of successive solvent extracts of *J. beddomei*. **Methodology:** Phytochemical screening, total phenolic, alkaloid and flavonoid contents were determined using standard methods. The antioxidant activity of plant extracts was determined by DPPH and ABTS scavenging assays. The antimicrobial activity of the plant extracts was determined by agar well diffusion method. **Results and Discussion:** The qualitative phytochemical screening results demonstrated that each extract (water, petroleum ether, chloroform, and ethyl acetate) was mainly constituted of phenols, flavonoids, and alkaloids. The greatest concentrations of phenolic (70.21 ± 0.4086 µg gallic acid equivalent/mg extract), flavonoids (23.36 ± 0.3007 µg quercetin equivalent/mg extract) and alkaloids (74.56 ± 0.5052 µg atropine equivalent/mg extract) were found in the ethanol extract. The extracts of water, petroleum ether, and chloroform of *J. beddomei* showed moderate inhibitory activity against *Staphylococcus aureus* (MTCC 87) at 1000 µg. None of the extracts exhibited any inhibitory effects on *Pseudomonas aeruginosa* (MTCC 741) up to 1000 µg. The ethyl acetate and water extract of *J. beddomei* exhibited antifungal activity against *Candida albicans* (MTCC 227) at 1000 µg. According to the antioxidant studies, the aqueous extract had the highest scavenging activity for ABTS (IC_{50} 373.83 µg/ml) and DPPH (IC_{50} 368.90 µg/ml). **Conclusion:** *J. beddomei* possesses various secondary metabolites with antibacterial, antifungal, and antioxidant properties.

Keywords: ABTS, *Candida albicans,* DPPH, *Justicia beddomei*, *Pseudomonas aeruginosa, Staphylococcus aureus*

1. Introduction

Traditional medicine from prehistoric human societies across the globe has shown, that plants are the best possible sources of the most potent pharmaceutical substances. These species are only found in the southern section of India's Western Ghats[1]. Different studies on the antibacterial qualities of various plants against different pathogens have been executed, developing substitute antimicrobial medications derived from plants[2]. In search of antimicrobials from plants, a variety of phytochemical extracts have been investigated. Various biological properties have been demonstrated by phytochemicals[3]. The morphological features of *J. beddomei* (C.B. Clarke) Bennet[4] (family Acanthaceae) is closely related to *Justicia adhathoda*. *J. adhathoda* showed antioxidant, antibacterial, cold, cough, asthma, and tuberculosis activities[5]. Traditionally, a variety of ailments have been treated with *J. beddomei* include fever, cough, leprosy, heart issues, blood disorders, hemorrhage and tuberculosis[6]. The literature shows that some studies have been performed on the quantitative estimation of phytoconstituents on ethanol extract of *J. beddomei*[7,8]. To the best of our knowledge, *J. beddomei* has not been explored for quantitative estimation of phytochemicals, antioxidant activities and antimicrobial screening.

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Phytochemicals are biological metabolites with nutritional value for human life that naturally occur in plants. These supplemental metabolites consist of steroid, flavonoids, alkaloids tannins, phenols, glycosides, gums, and terpenoids[9,10]. However, time, temperature, solvent concentration, and solvent polarity are typically influencing factors in the extraction and purification of phytochemicals from the plant material. It is not feasible to depend on a single solvent to precisely extract every phytochemical present in the plant material, so different phytochemicals are extracted in solvents with differing polarities depending on their chemical composition[11]. Reactive Oxygen Species (ROS) are created when plants are exposed to unfavourable environmental factors, which might result in oxidative damage. To stop oxidative stress, cells have an advanced antioxidant system that includes catalytical biochemical components. The non-catalytical biomolecules work in multiple ways, such as blocking enzyme activity, chelating trace elements that generate free radicals, absorbing and triggering reactive species and enhancing immunity against other antioxidant defenses[12]. The molecules produced by secondary metabolism, particularly the phenolic compounds are crucial in the fight against oxidative stress[13]. Because of their ability to donate electrons or hydrogen and their stability as intermediate radicals, these substances have a reputation for acting as antioxidants[14]. Human consumption of plant-based phenolic compounds provides protective benefits[15]. Due to the antioxidant property of phenol established lower risk of heart diseases and cancer in humans at low dosage[14-16]. These research activities may be a source of plant derived antioxidant compounds[17-19].

Researchers have studied the antimicrobial properties of *J. adhathoda* leaf extract both individually and in combination with other plant extracts as well. Bean mosaic virus was significantly inhibited by the plant's crude leaf extract[20]. Leaf extracts in chloroform and ethanol also demonstrated *in vitro* antifungal efficacy against systemic fungal infections[21]. *J. beddomei* is a plant that resembles morphologically with *J. adhathoda*. Thus, *J. beddomei* may also exhibit similar antimicrobial properties.

So, the phytochemical screening of *J. beddomei*, utilising different solvents with increasing polarities will not only help to reveal the components of whole plant extracts but also to extract the maximum amount of necessary phytochemicals that can be used in the discovery of more effective drugs for treating various diseases. Further research on *J. beddomei* will also take into account the antibacterial and antioxidant capabilities of the plant's numerous crude extracts.

2. Materials and Methods

2.1 Plant Material Collection

The *J. beddomei* (C.B. Clarke) Bennet plant was procured from the herb garden at the Arya Vaidya Sala in Kottakkal and verified by the Department of Forest Botany, Kerala Forest Research Institute, Peechi, Thrissur and maintained the plant specimen's herbarium with Accession Number 19373.

2.2 Chemical Reagents

The analytical grade chemical reagents were purchased from the Chemind, Kerala, India.

2.3 Preparation of Extracts and Preliminary Phytochemical Analysis

A 250 g powdered whole plant was placed inside a soxhlet apparatus, and successive extraction technique was performed using a range of solvents include petroleum ether, chloroform, ethyl acetate, ethanol and water. A rotary evaporator and lyophilizer was used to remove the solvent and preserved in a cool place for further experiment. Using standard procedures, a preliminary phytochemical screening was also performed on all the extracts[22-24].

2.4 Determination of Physicochemical Constants

The standard procedure was followed to determine the moisture content, extractive values, and ash values in triplicate[25].

2.5 Quantitative Evaluation of Phytochemicals

2.5.1 Estimation of Phenols

A test sample of 100 µl was pipetted out of a stock solution (10 mg/ml) and diluted with distilled water to 1.0 ml volume. Subsequently, 0.5 ml of folin-ciocalteu reagent and 2 ml of 20% Na_2CO_3 were added and boiled for a minute. The absorbance was measured at 750 nm against a blank, for the reagent, after cooling. Gallic acid as standard in solution, concentration ranging from 2.5 to 100 µg/ml, were also tested in the same way[26].

2.5.2 Estimation of Flavonoids

The colorimetric technique using aluminium chloride was used to determine the total flavonoid content. A reaction mixture comprising of 1 mg of extract and 4 ml of distilled water was taken and treated with 0.30 ml of 5% sodium nitrite and 0.30 ml of 10% aluminium chloride. Five minutes later, 2 ml of 1M sodium hydroxide was added and diluted to 10 ml with distilled water. Quercetin was used as reference standard and treated similar manner with different concentration (20, 40, 60, 80, and 100 µg/ml). The total flavonoid content was expressed as µg of quercetin equivalent per milligram of extract. The absorbance of the test and standard solutions was measured at 510 nm[26].

2.5.3 Estimation of Alkaloid

1 ml of 2N HCl was added to a solution of 1 mg of the plant extract in 1 ml of DMSO and the mixture was filtered. 5 ml each of phosphate buffer and bromocresol green solution were added and transferred to a separating funnel. The mixture was collected in a 10 ml volumetric flask and diluted to the volume with chloroform after being vigorously shaken with 1, 2, 3, and 4 ml of the chloroform. In the same way as previously mentioned, a series of reference standard solutions containing atropine (20, 40, 60, 80, and 100 µg) were also prepared and the absorbance was measured at 470 nm against the blank[27,28].

2.6 Antimicrobial Assay by Agar Well Diffusion Method

2.6.1 Antibacterial Assay

The test was carried out by a common method of diffusion of plant extract through prepared wells in the solidified agar plates[29]. Test samples of T1-50 µL and T2-100 µL were added to the wells from the 10 mg/ml stock. Control both positive and negative, gentamycin (40µl from a 4 mg/ml stock) and the sample dilution solvent were added, respectively. The plates were incubated in an aerobic environment at 36°C ± 1°C for 24 hours. The plates were examined after incubation, and the area around the wells where bacterial growth was inhibited was measured in millimetres.

2.6.1.1 Culture Media

Agar medium (HIMEDIA-M173) muller hinton agar measures organism's resistance to antimicrobial agents. 38 grams suspended in one thousand milliliters of boiling distilled water to dissolve the medium entirely. Autoclaved for 15 minutes at 121°C (15 pounds of pressure) to sterilize. Then was further cooled to 45–50 °C. After thoroughly mixing, transfer to sterile petri dishes.

2.6.1.2 Test Organisms

Staphylococcus aureus (MTCC 87) and *Pseudomonas aeruginosa* (MTCC 741) were used as the test organisms and were supplied by the Chandigarh-based Microbial Type Culture Collection (MTCC). *S. aureus*, a gram positive and *P. aeruginosa*, a gram negative are versatile bacterial pathogens that are frequently involved in polymicrobial infections in humans[30].

2.6.2 Antifungal Assay

The test was carried out using an equal proportion of a mixture of potato dextrose agar MH096 himedia and mueller-hinton agar. The test specimens (T1-50 µL and T2-100 µL) were added into the wells using the 10 mg/ml stock. A control of both positive and negative, 40µl of clotrimazole (300 µg/ml stock) and the sample dilution solvent was added, respectively. 48 hours of incubation were done on the plates at 27°C ± 1°C and the well-circumference zone of inhibition was measured in millimeters[31,32].

2.6.2.1 Culture Medium

The medium used was a 1:1 mixture of potato dextrose agar (MH096 himedia) and mueller-hinton agar. After heating the medium to 121°C (15 pounds of pressure), the ingredients were dissolved, and it was then autoclaved for 15 minutes. After that, it was cooled down to 45–50 °C. Transferred to sterile petri plates after well mixing.

2.6.2.2 Test Organisms

Fungal strain; *Candida albicans* (MTCC 227) was used in the study and was supplied by the Chandigarh-based Microbial Type Culture Collection (MTCC). *C. albicans* is easy to handle in the laboratory and can grow on standard yeast media without much problem[33]. The organism is responsible for most of the fungal infections in humans.

2.7 Antioxidant Activity

The antioxidant property was measured using ABTS (azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

radical scavenging assay and DPPH (1,1-diphenyl-2-picryl hydrazyl) by decolorization assay procedure[34].

Ascorbic acid was used as the reference standard in DPPH assay. With distilled water, an ascorbic acid stock solution (1 mg/ml; w/v) was prepared. Freshly prepared, 60µM DPPH in methanol solution was divided into 200µl and combined with 50 microliter of test sample to obtain different concentrations of 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1000 µg/ml. After placing in dark for 15 minutes, the absorbance at 515 nm was measured. DPPH solution and 95% methanol served as control and blank respectively. The experiment was run three times, and the percentage inhibition was determined[35].

In the ABTS assay, pH 4.5 acetic acid buffer was used to dissolve 96.02 mg of ABTS, which was subsequently diluted to 25 ml to create a stock solution I. There were 7 mM/L of ABTS solution. 66.24 mg of potassium persulfate ($K_2S_2O_8$) were dissolved in acetic acid buffer solution (pH 4.5) to create stock solution II, which was subsequently diluted to 100 ml. At 2.45 mmol/L, $K_2S_2O_8$ was present in the sample. To create the ABTS reaction solution, a working solution comprising 1 ml of stock solution I and 2.45 ml of stock solution II was combined and left for 12 to 16 hours at room temperature in the absence of light. Acetate buffer pH 4.5 was used to dilute the 2.55 ml ABTS reaction solution to 65 ml ABTS working solution, which was then stored for 30 minutes.

200 µl of diluted ABTS was added to 1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1000 µg/ml of various sample extract concentrations to start the reaction. 50 µl methanol was used as a control instead of the sample. Methanol acts as a substitute. Absorbance for all the samples was measured at 734 nm[36-38].

3. Results and Discussion

3.1 Phytochemical Screening and Percentage Yield

In order to identify the existence of components with therapeutic potential, the whole plant of *J. beddomei* were subjected to antibacterial, antifungal and antioxidant studies. Verifying the existence of phytochemicals in different *J. beddomei* preparations was the study's objective. Phytoconstituents in plants give them therapeutic properties. Qualitative screening indicated that alkaloids, flavonoids, and phenolic compounds were the main phytochemicals present in all of the extracts (Table 1). The percentage yield of constituent in each extract ranged from 0.39-3.96 %w/w and ethanol was good solvent from the yield report (Table 2).

Table 1. Phytochemical screening of *J. beddomei*'s different extracts

Phytochemical constituents	Chemical Test	PEJB	CHJB	EAJB	EJB	WJB
Alkaloid	Dragendorff's test	+	+	+	+	-
Flavonoids	Shinoda test	+	+	+	+	+
Glycosides	Picric acid	-	-	-	-	-
Phenol	Folin ciocalteau reagent	+	+	+	+	+
Saponins	Foam test	-	-	-	+	-
Tannins	Lead acetate	-	-	-	+	+
Terpenoids	Salkowski reaction	-	+	-	-	+
Carbohydrate	Molisch	+	+	+	+	+

'+' represents presence and '-' represents absence

PEJB: Petroleum ether extract of *J. beddomei*, CHJB: Chloroform extract of *J. beddomei*, EAJB: Ethyl acetate extract of *J. beddomei*, EJB: Ethanol extract of *J. beddomei*, WJB: Water extract of *J. beddomei*.

Table 2. Percentage yield and quantitative phytochemical screening of different extracts of *J. beddomei*

Extracts	Percentage yield (%w/w)	Concentration of Phenol in Gallic acid equivalent (µg/mg)	Concentration of Flavonoid in Quercetin equivalent (µg/mg)	Concentration of Alkaloid in Atropine equivalent (µg/mg)
PEJB	0.67	42.23±0.1710	4.88±0.0503	77.33±0.02
CHJB	0.39	54.68±0.5297	9.12±0.0503	71.78±0.4458
EAJB	3.96	68.11±0.5510	20.64±0.3842	90.11±0.4159
EJB	2.64	70.21±0.4086	23.36±0.3007	74.56±0.5052
WJB	1.19	41.79±0.4618	1.85±0.0503	55.11±0.2940

3.2 Physicochemical Parameters

Physical and chemical parameters reveal the plant's purity.

3.2.1 Ash Value

The percentages of total ash (7.05±0.5709 % w/w), ash that is soluble in water (0.002±0.001 % w/w) and insoluble in acid (1.803±0.0826 % w/w) which is less than the maximum plant standard ash value limit 10% w/w, in the dried aerial parts of *J. beddomei*.

The amount of material remaining after the powdered plant is ignited is known as the total ash value. Typically, silica, carbonates, phosphates, and silicates make up the majority of the total ash, which includes both non-physiologic and physiologic ash. The total value of ash in *J. beddomei* (7.05±0.5709 % w/w) indicates that adulteration, contamination and substitution in the plant is less. The low acid insoluble ash value (1.803±0.0826 % w/w) shows that there was no contamination of plant with sand and oil. Water soluble ash value (0.002±0.001 % w/w) demonstrates the information regarding the water-soluble salt of the selected drug. The low concentration of water-soluble salt in the selected plant, acid ash value that is insoluble, and the total ash content, all supported purity of *J. beddomei*.

3.2.2 Extractive Value

The presence of polar or non-polar extractable compounds in a plant material is indicated by the extractive values. From the extractive values, the ethanol extract showed the maximum value (27.86±0.0451 % w/w) and the presence of more polar chemical constituent than non-polar constituent. The ethyl acetate extract showed the minimum extractive value (13.26±0.0153 %w/w). The extractive value of chloroform extract of *J. beddomei* is 17.48±0.0458 %w/w followed by petroleum ether extract (16.22±0.0379 %w/w) and water extract (15.16±0.0361 % w/w).

Crude drugs will always contain moisture, so it is best to get rid of it as much as possible. Inadequate drying cause mould and bacteria growth and allows for the enzymatic destruction of active principles. The total dried plant's moisture content was found to be 10.384 ± 0.0399 % w/w. The moisture content revealed the presence of minimum amount of water in the plant.

3.3 Quantitative Phytochemical Analysis

The quantitative phytochemical screening of phenol, flavonoid and alkaloid in the different solvent extracts was performed (Table 2). The ethanol extract of *J. beddomei* had the highest total phenolic content of 70.21±0.4086 µg of gallic acid equivalents per milligram of plant extract and total flavonoid content of 23.36±0.3007 µg of quercetin equivalents per milligram of plant extract than other extracts. The petroleum ether extract of *J. beddomei* had the highest total alkaloid content of 77.33±0.02 µg of atropine equivalents per milligram of plant extract than other extracts.

3.4 Antimicrobial Assay by Agar Well Diffusion Method

3.4.1 Antibacterial Assay

Different solvent extracts of entire *J. beddomei* plant at two concentrations 500 µg and 1000 µg displayed different zones of inhibition and were compared with the Gentamycin standard (160µg) (Table 3). Two bacterial strains were used to test the extracts antibacterial qualities: The gram-positive, *S. aureus* and the gram-negative, *P. aeruginosa*. At 1000 µg, petroleum ether (PEJB), chloroform (CHJB), and water extract (WJB) of the plant demonstrated activity against *S. aureus* at 9 mm, 10 mm, and 9 mm, respectively, at 500 µg, no activity was observed. The 500 µg and 1000 µg concentrations of ethanol (EJB) and ethyl acetate extract (EAJB) were inert against *S. aureus*. At 500 µg and 1000 µg, the chosen concentrations of *J. beddomei* extracts did not exhibit any activity against *P. aeruginosa*. The extracts phenolic compound content may be the cause of their antibacterial activity against various bacterial strains. However, due to variations in compositions other than phenolic content in the extracts there were differences in activities. Thus, in addition to phenolic compounds, the contents in complex extracts affected their antibacterial properties. Although ethyl acetate and ethanol extracts contained a certain quantity of total polyphenols but did not show antibacterial activity towards *S. aureus*. In contrast, petroleum ether, chloroform and water extracts containing a low quantity of phenol showed activity towards *S. aureus*. The presence of phytoconstituents influenced the inhibition activity of all the extracts towards *P. aeruginosa*.

Table 3. Antibacterial activities of different extract of *J. beddomei* against bacterial test organisms

Sl. No.	Name of Microorganism	Sample code	Zone of inhibition (mm)			
			Standard Gentamycin (160 µg)	Negative control	T_1 (500µg)	T_2 (1000µg)
1	*S. aureus*	PEJB	24mm	-	-	9mm
2		CHJB	24mm	-	-	10mm
3		EAJB	24mm	-	-	-
4		EJB	24mm	-	-	-
5		WJB	24mm	-	-	9mm
6	*P. aeruginosa*	PEJB	21mm	-	-	-
7		CHJB	21mm	-	-	-
8		EAJB	21mm	-	-	-
9		EJB	21mm	-	-	-
10		WJB	21mm	-	-	-

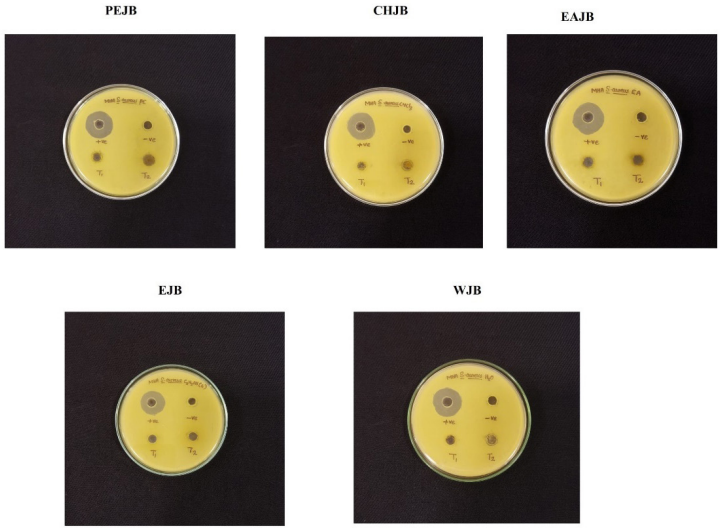

Figure 1. Zone of inhibition of *J. beddomei* against *S. aureus.*

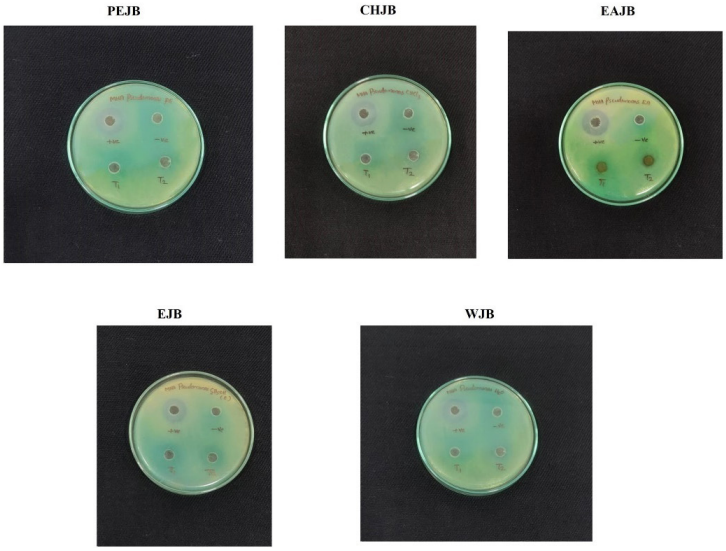

Figure 2. Zone of inhibition of *J. beddomei* against *P. aeruginosa.*

3.4.2 Antifungal Assay

In vitro antifungal properties of various solvent-extracted whole plant preparations at two concentrations, 500µg and 1000µg of *J. beddomei* were studied against *Candida albicans* and compared with the standard Clotrimazole at 120 µg (Table 4). Ethyl acetate and water extract showed activity (9mm inhibition zone) in opposition to *C. albicans* at 1000µg concentration. The other extracts petroleum ether, chloroform and water did not show any activity at both concentrations. The extracts antifungal properties could be attributed to the presence of alkaloids as suggested by scientific research and petroleum ether, chloroform and water extracts biological activity against fungus could be attributed to the presence of alkaloids.

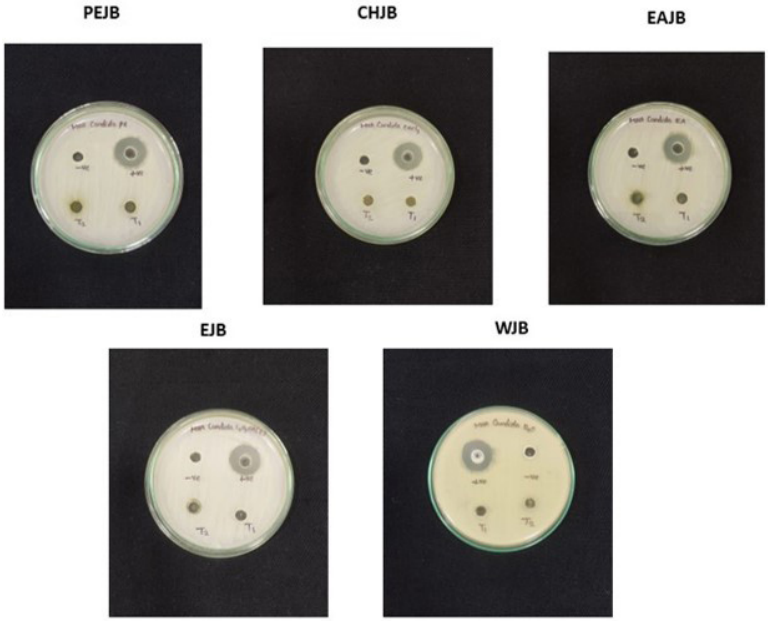

Figure 3. Zone of inhibition of *J. beddomei* against *C. albicans.*

Table 4. Antifungal activities of different extract of *J. beddomei* against fungal test organism

Sl. No.	Name of Microorganism	Sample code	Standard Clotrimazole (120 µg)	Negative control	T_1 (500µg)	T_2 (1000µg)
1	*Candida albicans*	PEJB	20 mm	-	-	-
2		CHJB	20 mm	-	-	-
3		EAJB	20 mm	-	-	9 mm
4		EJB	20 mm	-	-	9 mm
5		WJB	20 mm	-	-	-

3.5 Antioxidant Activity

Tables 5, 6 and Figure 4 show outcomes of the test for scavenging DPPH and ABTS radicals. Using *J. beddomei* extracts resulted in an increase in the percentage antioxidant activity that was concentration-dependent.

The WJB's ability to scavenge free radicals in terms of DPPH showed minimum IC_{50} 368.90 µg/ml value. The IC_{50} values for PEJB, CHJB, EAJB, and EJB extracts exhibited IC_{50}values of 853.76 µg/ml, >1000 µg/ml, 985.69 µg/ml, and >1000 µg/ml, respectively (Table 7).

The ABTS radical scavenging assay results are displayed in Table 6 and Figure 5. A concentration-dependent rise in the percentage of antioxidant activity was observed in *J. beddomei* extracts with varying polarity bases. Based on the findings, the minimum IC_{50} values for both CHJB and WJB extracts were 300.33 µg/ml and 373.84 µg/ml respectively. PEJB, EAJB, EJB extracts had IC_{50} values of 503.50, 972.66, and 672.05 µg/ml, respectively. The presence of the phenolic compounds in the extracts might be responsible for the antioxidant property (Table 7).

Table 5. Antioxidant activity of different extract of *J. beddomei* using DPPH radical scavenging assay

Concentration (µg/ml)	Mean Percentage antioxidant activity±SD					
	Std (Ascorbic acid)	PEJB	CHJB	EAJB	EJB	WJB
1.56	3.36±0.76	3.68±0.04	1.35±0.03	0.66±0.01	2.69±0.04	4.65±0.01
3.12	11.61±0.04	6.36±0.01	3.24±0.04	3.13±0.02	4.72±0.04	9.43±0.03
6.25	17.22±0.01	8.01±0.02	6.14±0.04	4.97±0.05	8.41±0.02	14.77±0.05
12.5	32.98±0.02	18.14±0.04	8.73±0.04	7.72±0.03	12.23±0.02	17.54±0.02
25	44.26±0.04	23.17±0.02	13.37±0.03	10.96±0.04	15.92±0.03	23.54±0.04
50	82.19±0.01	29.36±0.03	16.41±0.04	15.94±0.03	21.28±0.04	29.41±0.03
100	88.75±0.04	33.19±0.03	20.49±0.02	19.91±0.03	29.42±0.03	33.32±0.02
200	89.75±0.05	38.10±0.04	23.28±0.03	27.32±0.04	35.22±0.03	40.79±0.05
400	92.11±0.04	41.69±0.04	26.63±0.02	30.76±0.01	39.27±0.02	51.54±0.04
800	94.95±0.04	48.25±0.02	29.98±0.01	37.77±0.02	45.59±0.03	60.06±0.03
1000	97.24±0.03	53.30±0.02	33.45±0.05	45.35±0.01	50.83±0.03	70.08±0.03

Table 6. Antioxidant activity of different extract of *J. beddomei* using ABTS radical scavenging assay

Conc. (µg/ml)	Mean Percentage antioxidant activity ± SD					
	Std (Ascorbic acid)	PEJB	CHJB	EAJB	EJB	WJB
1.56	8.20±0.01	1.96±0.01	3.51±0.01	1.69±0.01	4.86±0.02	3.40±0.02
3.12	14.80±0.02	6.05±0.03	7.48±0.05	5.62±0.04	9.63±0.03	7.46±0.01
6.25	27.58±0.02	7.90±0.04	13.93±0.05	10.54±0.04	12.77±0.02	12.22±0.02
12.5	43.04±0.03	11.97±0.05	16.32±0.25	13.95±0.05	16.45±0.05	18.04±0.06
25	56.42±0.05	17.77±0.01	22.66±0.26	16.61±0.05	26.26±1.39	26.34±0.03
50	76.73±0.07	25.96±2.28	27.28±0.01	22.54±0.04	28.59±0.03	31.22±0.06
100	90.48±0.02	26.84±0.09	32.22±0.22	25.43±0.03	33.23±0.19	35.98±0.04
200	94.72±0.03	41.51±4.53	47.11±0.03	29.79±0.02	14.24±10.85	44.07±0.29
400	96.10±0.03	47.85±0.02	53.26±0.16	36.48±0.01	42.75±0.14	50.49±0.03
800	96.40±0.02	57.66±0.11	60.21±0.03	43.74±0.02	51.53±1.47	58.77±0.02
1000	97.24±0.03	59.95±0.05	65.93±0.04	52.05±0.03	61.05±0.04	70.20±0.14

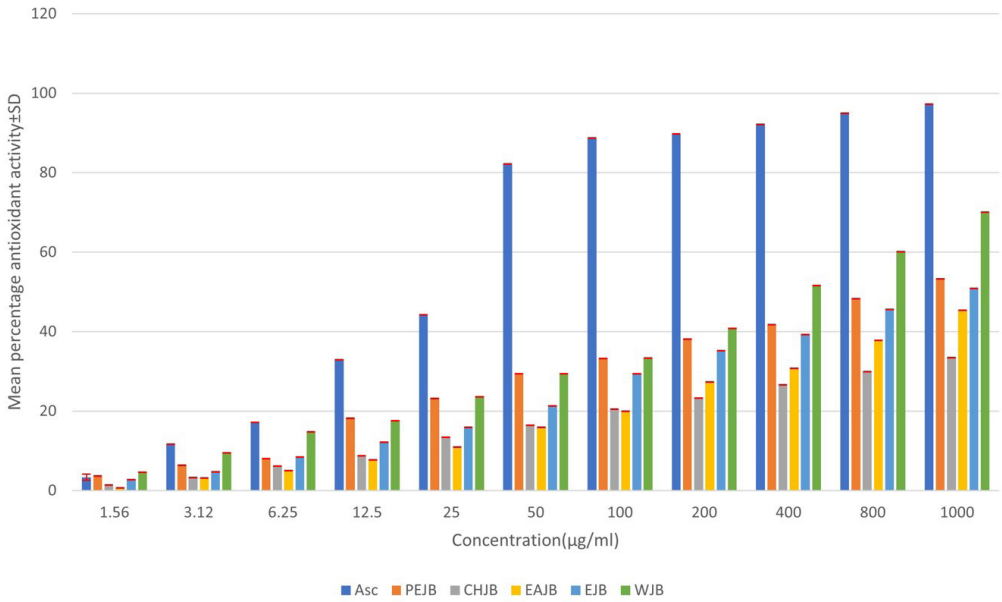

Figure 4. DPPH radical scavenging assay of *J. beddomei.*

Table 7. IC_{50} values of DPPH and ABTS radical scavenging assays of *J. beddomei*

Compound Name	DPPH radical scavenging assay IC_{50} (µg/ml)	ABTS radical scavenging assay IC_{50} (µg/ml)
Ascorbic acid	28.19	19.69
PEJB	853.76	503.50
CHJB	>1000	300.33
EAJB	>1000	972.66
EJB	985.69	672.05
WJB	368.90	373.84

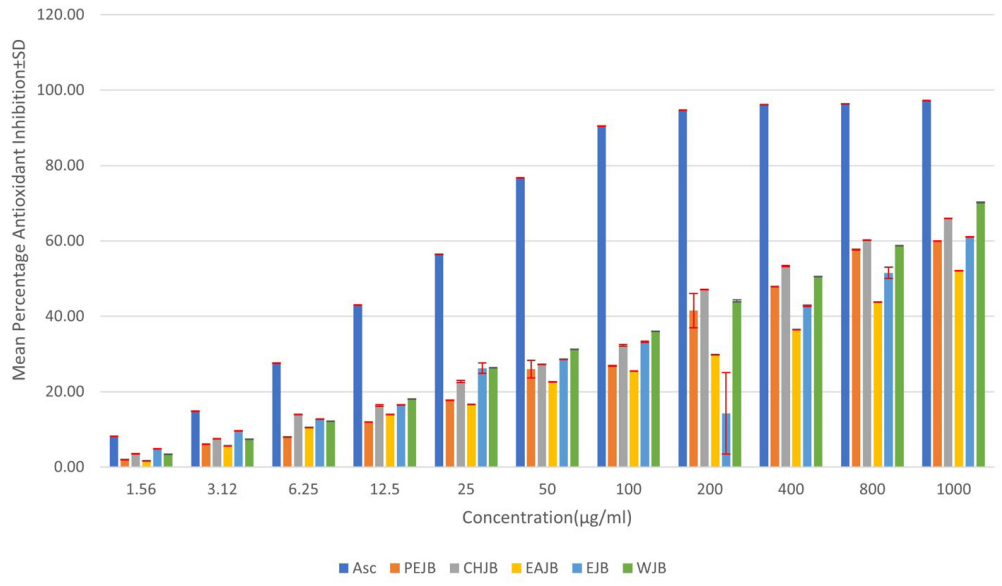

Figure 5. ABTS radical scavenging assay of *J. beddomei.*

Both the methods (DPPH and ABTS) have shown that water extract exhibits good antioxidant properties followed by the chloroform extract of the plant *J. beddomei*.

4. Conclusion

Secondary metabolites, alkaloids, phenols, and flavonoids were found to be significantly present in *J. beddomei* extracts through qualitative screening. The results of the physicochemical parameter analysis demonstrated the selected plant's purity due to its low moisture content, total ash value and the extractive value revealed ethanol as the best solvent. EJB contains the highest content of total phenolics, 70.21±0.4086 µg gallic acid equivalent/mg dry weight and total flavonoids 23.36±0.3007 µg quercetin equivalent/mg dry weight than other extracts. The PEJB contains the highest amount of alkaloids 74.56±0.5052 µg atropine equivalent/mg dry weight in comparison with other extracts. Antibacterial activity of *J. beddomei* extracts (PEJB, CHJB and WJB) shows good activity against *S. aureus* at 1000µg. The extracts did not exhibit antibacterial activity against *P. aeruginosa* upto 1000µg. At 1000 µg, WJB and EAJB demonstrated antifungal activity against *C. albicans*. With IC_{50} values of 368.90 µg/ml and 373.83 µg/ml, respectively, the DPPH and ABTS scavenging assay values for the aqueous extract, the antioxidant property were the highest. It is possible to draw conclusions from the results that *J. beddomei* contains active secondary metabolites with antibacterial, antifungal and antioxidant properties.

5. References

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