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Research Article

Phytochemical Analysis and Antioxidant Evaluation of a Crude Leaves Extract of Nerium Indicum (Mill)

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ABSTRACT

Nerium indicum (Mill) is a well-known medicinal plant with a rich history of use in Ayurvedic and traditional medicine for treating various ailments. This study investigates the phytochemical composition and antioxidant potential of Nerium indicum leaf extracts using methanol and water as solvents. Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, steroids, and tannins, with methanol extracts showing a more substantial presence of bioactive compounds than water extracts. The antioxidant activity was evaluated using the DPPH radical scavenging assay, where the methanol extract exhibited superior activity (96.179 ± 3.228% inhibition at 400 µg/mL) compared to the water extract (81.097 ± 0.234%). The IC50 value of the methanol extract (30.218 ± 2.957 µg/mL) was lower than that of the water extract (33.209 ± 0.487 µg/mL), indicating higher antioxidant potential. These findings suggest that Nerium indicum is a potent source of natural antioxidants, which could be beneficial in managing oxidative stress-related diseases. The plant's phytochemical profile and radical solid scavenging activity support its traditional use and highlight its potential for pharmaceutical applications.

Keywords: Antioxidant, DPPH radical scavenger, Evaluation, Nerium indicum (Mill), Phytochemical

INTRODUCTION

The medicinal use of plants has been integral to human health for millennia, evolving alongside human history. Plants have long been recognized for their rich reservoir of secondary metabolites, which play crucial roles in pharmacology and drug discovery. Among these secondary metabolites, polyphenols stand out due to their potent bioactive properties. Polyphenols are a diverse group of phytochemicals that exhibit potent antioxidant, antimicrobial, anti-inflammatory, and antitumor activities (Munin & Edwards-Levy, 2011). They are known to offer vascular protection, with tannins, anthraquinones, and flavonoids being particularly effective in providing these benefits (Xia*et al.,* 2010). The antioxidant properties of polyphenols are especially notable for their ability to mitigate oxidative stress, a condition linked to the overproduction of reactive oxygen species (ROS) such as superoxide (O_2) and hydrogen peroxide (H_2O_2), which damage cellular structures and biological molecules.

Oxidative stress is implicated in the pathogenesis of numerous diseases, including cardiovascular diseases, cancer, and neurodegenerative disorders. The body relies on several endogenous defence mechanisms to counteract oxidative stress, such as enzymatic antioxidants, chelating proteins, and transition metals (Perez-Matute*et al.,* 2012). However, exogenous antioxidants, particularly those derived from plant sources like polyphenols, are increasingly recognized for their protective effects. These compounds neutralize free radicals, such as hydroxyl (OH) and superoxide radicals, by donating electrons, thereby preventing cellular damage (Popovici *et al.,* 2009).

Given the growing incidence of oxidative stress-related diseases, natural antioxidants from medicinal plants represent a promising area of research for developing therapeutic agents.

Nerium indicum (oleander) is a medicinal plant traditionally used in the Mediterranean and Asian regions to treat various ailments. Ethnobotanical studies have shown that *Nerium indicum* is employed in the treatment of ulcers and inflammation, and even for inducing abortions (Hseini & Kahouadji, 2007). Its leaves are used in poultices for conditions such as itching, hair loss, lice infestations, and toothaches, as well as for managing diabetes (Lahsissene *et al.,* 2009). This widespread use emphasizes its potential pharmacological value, though comprehensive scientific studies exploring its bioactive components and mechanisms of action are still needed.

Nerium indicum belongs to the Apocynaceae family and is widely recognized for its ornamental and medicinal properties. It is an evergreen shrub or small tree with glossy, lanceolate leaves and fragrant flowers that vary in colour from red to white and pink (Singh *et al.,* 2010). While cultivated worldwide, particularly in Southwest Asia, *Nerium indicum* is native to India, Bangladesh, Nepal, Myanmar, and China. Despite its toxic components notably in the roots and leaves the plant has been extensively used in traditional medicine for centuries. The toxicity, mainly due to cardiac glycosides, demands careful processing before its medicinal application (Mokkhasmit *et al.,* 1971).

Scientific classification places *Nerium indicum* within the kingdom Plantae, division Tracheophyta, and class Magnoliopsida. As a member of the order Gentianales, it shares taxonomic characteristics with other medicinal plants in the family Apocynaceae. The plant's widespread use in traditional healing systems and its rich phytochemical composition may harbour untapped medicinal potential. However, there remains a pressing need to scientifically validate the traditional uses of *Nerium indicum* and explore its therapeutic efficacy, particularly in modern medical contexts where oxidative stress and inflammation are key targets (Rohini *et al.,* 2015).

By systematically analyzing the plant's bioactive components and evaluating its antioxidant potential using advanced biochemical assays, this research aims to contribute to the growing body of evidence supporting the medicinal use of *Nerium indicum*. This study focuses on extracting phytochemicals from the leaves and testing their antioxidant capacity using the DPPH radical scavenging assay to identify compounds that could be developed into natural therapeutic agents for oxidative stress-related conditions.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plant parts, specifically leaves, were collected from medicinal gardens located in different regions of India. The selection of plants was based on their traditional medicinal use, with careful consideration of their therapeutic properties. Information regarding each plant's family, species, and common names was documented during the collection process. Certified plant taxonomists authenticated the taxonomic identities of the plants, and voucher specimens were prepared and deposited in the herbarium for future reference. The voucher specimen numbers were recorded for each plant species to ensure accurate identification and traceability.

After collection, the fresh plant materials were thoroughly washed with tap water to remove any adhering dirt or contaminants. After cleaning, the plant materials were allowed to air-dry under shade at room temperature for 7-10 days. This ensured that the phytoconstituents were preserved without direct sunlight, which could degrade sensitive compounds. Once dried, the plant materials were ground into a fine powder using a mechanical grinder. The homogenized powder was sieved to achieve uniform particle size and was stored in airtight containers to prevent moisture absorption and contamination. These containers were labelled and kept in a cool, dry place until further use in downstream experimental procedures.

Extraction of Plant Material

Ten grams of powdered leaves of *Nerium indicum* were accurately weighed and transferred into a conical flask. Two solvents, methanol and water, were selected based on their polarity and extraction efficiency to ensure the comprehensive recovery of phytochemicals. For each solvent, 25 mL was added to the powdered plant material. The flasks were labelled correctly and subjected to Soxhlet extraction for 48 hours. Soxhlet extraction was chosen due to its efficiency in continuously extracting bioactive compounds over an extended period without the need for constant replenishment of solvent. After the extraction period, the mixture was cooled to room temperature. The extract was

filtered using Whatman No. 1 filter paper to remove particulate matter, ensuring a clean filtrate. The filtered extract was collected in pre-weighed, labelled containers. To concentrate the extract, the filtrate was evaporated to dryness using a water bath set at an appropriate temperature (below 40°C) to avoid the degradation of thermolabile compounds. This process produced a semi-solid mass, which was further dried to remove residual moisture and solvent.

The dried extract was weighed and transferred into airtight containers, which were properly sealed and labelled. To preserve the integrity and stability of the phytochemicals for future analysis, the containers were stored at 4°C in a refrigerator. This storage condition is crucial for maintaining the bioactivity of the compounds by preventing oxidative degradation and microbial growth during prolonged storage (Sasidharan *et al.,* 2011).

Phytochemical Analysis

To determine the presence of various phytochemical constituents, such as alkaloids, tannins, steroids, saponins, cardiac glycosides, flavonoids, and phenols, phytochemical analysis was performed using different solvents, namely methanol and water. The analysis involved subjecting the plant extracts to a series of well-established qualitative tests.

To detect alkaloids, the leaf extracts of *Nerium indicum* were heated in a water bath until completely dry. The residue was then dissolved in 2N hydrochloric acid (HCl), and the resulting filtrate was divided into three portions. Mayer's reagent, Dragendorff's reagent, and Wagner's reagent were added separately to each portion. The formation of a cream-coloured precipitate with Mayer's reagent indicated the presence of alkaloids. In contrast, an orange precipitate with Dragendorff's reagent and a brown precipitate with Wagner's reagent confirmed the presence of other alkaloid types (Salehi-Surmaghi *et al.,* 1992).

Flavonoid content was determined using the Shinoda test. A small magnesium ribbon was added to the rhizome extract, followed by a few drops of concentrated HCl. The development of a pink or tomato-red colour after a few minutes was taken as a positive indication of flavonoids (Somolenski *et al.,* 1972). To detect tannins, the rhizome extract was treated with an alcoholic ferric chloride ($FeCl_3$) solution. The appearance of a blue colour suggested the presence of tannins (Segelman *et al.,* 1969).

The presence of cardiac glycosides was determined using the Keller-Kilian test. A mixture of 1 mL of a 5% $FeCl_3$ solution in glacial acetic acid was added to the powdered plant material, followed by a few drops of concentrated sulfuric acid (H_2SO_4). The formation of a greenish-blue colour indicated cardiac glycosides (Ajaiyeobu, 2002). Steroids were tested by adding concentrated sulfuric acid to the crude leaf extracts. The development of a yellow colour with green fluorescence in the sulfuric acid layer and a red supernatant indicated the presence of steroids.

The Frothing test was used to identify saponins. The dry powdered leaves were shaken vigorously with distilled water and observed for froth formation. The presence of stable foam greater than 1.5 cm in height after 10 minutes indicated the presence of saponins (Kapoor *et al.,* 1969). A test was performed for phenols by adding 5 drops of 10% ferric chloride to 1 mL of the extract in 2 mL of distilled water. The formation of a blue or green colour confirmed the presence of phenols. These methods, widely accepted in phytochemical screening, ensured the identification of critical bioactive compounds in the *Nerium indicum* extracts, vital for understanding the plant's potential therapeutic properties.

Evaluation of the Antioxidant Activity of *Nerium indicum*

The detection of anti-radical substances in the plant extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, following the method described by Sgherri *et al.* (2012). This method is widely used to evaluate the antioxidant capacity of plant extracts based on their ability to scavenge free radicals. In this assay, DPPH, a stable free radical, is reduced by antioxidants in the sample, leading to a decrease in absorbance, which can be quantitatively measured.

A range of extract concentrations (100 to 400 µg/mL) was prepared to begin the assay. A total of 900 µL of a freshly prepared DPPH solution (0.004% w/v in methanol) was added to 100 µL of each extract in test tubes, resulting in a total reaction volume of 1 mL. After thorough mixing, the test tubes were covered with aluminium foil to prevent light-induced degradation of the DPPH reagent and incubated in the dark at room temperature for 30 minutes.

This incubation time allows sufficient interaction between the antioxidants present in the extract and the DPPH free radicals.

Following incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV-Vis spectrophotometer. Methanol was used as the blank. The decrease in absorbance at 517 nm indicates the extract's DPPH radical scavenging activity. The control sample consisted of the DPPH solution without any extract, and its absorbance was used as the reference for maximum radical concentration. The percent inhibition of DPPH free radicals by the plant extract was calculated using the following formula:

$$\% = \frac{(\text{Absorbance of control} - \text{Absorbance of the sample})}{(\text{Absorbance of control})} \times 100$$

Where:

- The absorbance of control is the absorbance of the DPPH radical solution mixed with methanol (no extract).
- The absorbance of the sample is the absorbance of the DPPH radical solution mixed with the plant extract or standard.

The antioxidant activity of the extracts was further quantified by determining the IC50 value, which is the concentration of the extract required to inhibit 50% of the DPPH radicals. The IC_{50} value was calculated by plotting the percent inhibition against the concentration of the extract and determining the point where 50% inhibition occurred. A lower IC_{50} value indicates higher antioxidant activity, reflecting the extract's ability to scavenge free radicals at a lower concentration (Blois, 1958; Brand-Williams *et al.,* 1995).

Statistical Analysis

All experimental results are expressed as mean ± standard deviation (SD) based on data obtained from at least three independent replicates for each assay. Statistical analysis was performed using one-way analysis of variance (ANOVA) to assess differences between the experimental groups. The analysis was conducted using SPSS software version 20.0 for Windows. ANOVA was chosen as it allows for the comparison of means across multiple groups, determining whether significant differences exist between them based on the variance within and between the groups. Mean values are considered significantly different if $p \leq 0.05$.

RESULTS AND DISCUSSION

Nerium indicum Leaf Extracts Yield in Different Solvents

The percentage yield of *Nerium indicum* leaf extracts was determined using methanol and water as extraction solvents, yielding 1.5% and 1.0% for methanol and water, respectively. The methanol extract produced a dark green paste, while the water extract produced a light green paste (Table 1). These differences in yield and extract characteristics can be attributed to the varying solubility of the phytochemicals in polar solvents such as methanol and water. Methanol, a moderately polar solvent, effectively extracts a wide range of polar and non-polar compounds, including alkaloids, flavonoids, and phenolics, which are typically abundant in plant materials like *Nerium indicum* (Wong *et al.,* 2014). In contrast, water, a highly polar solvent, primarily extracts hydrophilic compounds, leading to a lower yield.

Table 1: The percentage yield of solvent extracts of *Nerium indicum* (Leaves)

S/No.	Solvent	Weight of dried extract (g)	Yield (%)	Colour	Consistency
1.	Methanol	15	1.5	Dark green	Paste
2.	Water	10	1.0	Light green	Paste

The yield of *Nerium indicum* in methanol (1.5%) is consistent with previous studies on other medicinal plants, demonstrating that methanol generally produces higher extraction yields than water due to its ability to dissolve a broader spectrum of bioactive compounds (Azwanida, 2015). *Azadirachta indica* (neem) leaves have been shown to produce higher yields with methanol, similar to the findings in the current study. The relatively lower yield of 1.0% in water is typical for plant extractions using aqueous solvents, as water primarily extracts polar compounds, such as tannins and polysaccharides, but may be less effective for other important phytochemicals like alkaloids and flavonoids (Abd Razak *et al.,* 2012). The higher yield obtained with methanol suggests it is a more efficient solvent for extracting the bioactive compounds from *Nerium indicum*, potentially indicating the presence of methanol-soluble compounds like alkaloids, flavonoids, and phenolics. These compounds are known for their therapeutic properties, including antioxidant, anti-inflammatory, and anticancer activities (Chen *et al.,* 2013). Therefore, the methanol extract may have a broader spectrum of bioactivity due to the higher yield and potential presence of diverse phytochemicals.

Although yielding a lower percentage, the water extract is valuable in traditional medicine, where aqueous extracts are commonly used. The hydrophilic compounds extracted from water, such as polysaccharides, may significantly enhance immune responses or act as antioxidants (Altemimi *et al.,* 2017). This highlights the importance of solvent choice based on the intended use of the extract, whether for pharmacological studies or traditional applications.

The difference in extraction yield between methanol and water can be explained by the principle of "like dissolves like." Methanol's intermediate polarity allows it to extract a broader range of compounds, while water, being highly polar, is limited to extracting polar molecules like tannins, glycosides, and some phenolic acids. This aligns with the findings of Harborne (1998), who stated that solvents with intermediate polarity, such as methanol, are superior for extracting a wide variety of phytochemicals, especially non-polar and semi-polar compounds.

Comparing this with other medicinal plants, a study by Siddhuraju and Becker (2003) demonstrated similar results in extracting antioxidants from *Moringa oleifera* leaves, where methanol outperformed water in terms of yield and phytochemical content. This suggests that methanol is a generally preferred solvent for maximizing phytochemical recovery, mainly when targeting bioactive compounds for pharmaceutical applications.

Phytochemical Analysis

The phytochemical screening of the leaf extracts of *Nerium indicum*, using methanol and water as solvents, revealed the presence of several bioactive compounds with potential therapeutic significance. Both methanolic and aqueous extracts observed a strong presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, and cardiac glycosides. However, the distribution and intensity of these compounds varied between the two solvents (Table 2). Specifically, alkaloids were present in methanol and water extracts, with a high quantity presence observed in the extract (++), suggesting that water may be a more effective solvent for extracting this class of compounds. In contrast, cardiac glycosides were only detected in the methanol extract (++), indicating that these compounds are more soluble in moderately polar solvents like methanol, which is consistent with their semi-polar nature.

Table 2: Phytochemical Screening of Methanol and Water Extracts of *Nerium indicum*

S/No.	Phytochemical Components	Names of Reagents Used	Name of Extracts	
			Methanol	**Water**
1	Alkaloids	Mayers test	+	++
		Dragondroff's test	++	+
		Wagner's test	-	+
2	C. glycosides	Keller-kiliani's test	++	+
3	Flavonoids	Shinoda test	++	+
4	Saponins	H_2SO_4 test	-	+

5	Steroids	Frothing test	+	+
6	Tannins	Ferric chloride test	+	++
7	Phenols	$FeCl_3$	+	+

Note: (+++) - Strongly positive (++) - positive

(+) - Trace (–) - Not detected

The strong presence of alkaloids in both methanol and water extracts suggests that *Nerium indicum* contains significant amounts of these nitrogen-containing compounds known for their wide range of pharmacological activities, including analgesic, anti-inflammatory, and antimicrobial properties (Cordell, 1981). The more substantial presence of alkaloids in the water extract aligns with findings from studies on other medicinal plants like *Catharanthus roseus*, where water was shown to extract alkaloids effectively (Sasidharan *et al.,* 2011). This suggests that the traditional use of water-based preparations of *Nerium indicum* in herbal remedies may be justified for alkaloid-based treatments.

Cardiac glycosides, absent in the water extract but present in the methanol extract, are known for their role in treating heart failure and other cardiovascular conditions (Wichtl, 2004). The selective extraction of cardiac glycosides by methanol highlights its suitability for isolating semi-polar compounds, consistent with previous reports where methanol proved an efficient solvent for extracting cardiac glycosides from *Digitalis* species (Houghton *et al.,* 1995). This finding suggests that methanol extracts of *Nerium indicum* could be explored for their potential cardiotonic properties.

Flavonoids, which are present in both methanolic (++) and aqueous (+) extracts, are widely recognized for their antioxidant, anti-inflammatory, and anticancer activities (Middleton *et al.,* 2000). The more substantial presence of flavonoids in the methanol extract is consistent with previous studies, such as those conducted on *Moringa oleifera* and *Ocimum sanctum*, where methanol was found to be a superior solvent for flavonoid extraction (Kumar *et al.,* 2012). This supports the hypothesis that the methanol extract of *Nerium indicum* may have higher antioxidant potential due to its rich flavonoid content.

Tannins, which were detected more strongly in the water extract (++) compared to the methanol extract (+), are polyphenolic compounds known for their antimicrobial and astringent properties (Scalbert, 1991). The more substantial presence of tannins in water is consistent with their high solubility in polar solvents, as seen in previous studies on tannin extraction from *Camellia sinensis* (green tea) (Hagerman *et al.,* 1998). This implies that water-based extracts of *Nerium indicum* could be effective for applications that rely on tannins, such as wound healing and antimicrobial formulations.

Saponins, detected in the water extract but absent in the methanol extract, are glycosides known for their surface-active properties and are often associated with immune-modulatory and anti-inflammatory effects (Francis *et al.,* 2002). The presence of saponins in the water extract aligns with findings from studies on *Glycyrrhiza glabra* (liquorice), where water was more effective than methanol in extracting these compounds (Liu *et al.,* 2004). This suggests that aqueous extracts of *Nerium indicum* hold potential for immunomodulatory applications.

Detecting multiple bioactive compounds in *Nerium indicum* extracts highlights the plant's potential as a source of natural therapeutics. The presence of alkaloids, cardiac glycosides, flavonoids, and phenols in methanolic extracts suggests that methanol could be the preferred solvent for extracting compounds with antioxidant, cardioprotective, and anti-inflammatory properties. On the other hand, the presence of tannins and saponins in the water extracts indicates that water-based extracts could be more suitable for antimicrobial and immune-boosting applications.

These findings highlight the importance of solvent selection in phytochemical extraction, as different solvents target different classes of compounds. The strong presence of alkaloids, flavonoids, and phenols suggests that *Nerium indicum* may have broad therapeutic applications, including antioxidant, antimicrobial, and cardiotonic uses. Further pharmacological studies are needed to validate these activities *in vivo* and clinical settings.

The results of this study are consistent with previous reports on the phytochemical composition of medicinal plants. A study on *Nerium oleander*, a close relative of *Nerium indicum*, revealed a similar phytochemical profile, with alkaloids, flavonoids, and cardiac glycosides being abundant in methanolic extracts (Mahmood *et al.,* 2014).

Similarly, saponins and tannins in aqueous extracts of *Nerium indicum* mirror the findings in studies on other medicinal plants like *Tribulus Terrestris* (Zheleva-Dimitrova *et al.,* 2011).

The phytochemical profile observed in *Nerium indicum* also aligns with the World Health Organization's (WHO) emphasis on the potential of medicinal plants as sources of new drugs, particularly in the context of traditional medicine. According to the WHO, about 80% of the population in developing countries relies on plant-based medicines for primary healthcare (WHO, 2021). This reinforces the need for further research into the bioactivity and therapeutic potential of *Nerium indicum* extracts.

DPPH Radical Scavenging Capacity of *Nerium indicum*

The DPPH radical scavenging capacity of *Nerium indicum* leaf extracts was evaluated using methanol and water as solvents, and the results are presented in Table 3. The scavenging activity was concentration-dependent, with inhibition percentages increasing with the concentration of the extracts. At the highest 400 µg/mL concentration, the methanol extract exhibited 96.179±3.228% inhibition, while the water extract showed 81.097±0.234% inhibition. These values are comparable to ascorbic acid, a standard antioxidant with 99.472±0.026% inhibition at the same concentration.

Table 3: DPPH radical scavenging capacity of leaf extracts of *Nerium indicum*

S/No.	Concentration of extract (µg/mL)	Inhibition (%)		
		Methanol extracts	Water extracts	Ascorbic acid (std)
1	100	74.604±0.322	66.791±0.097	95.880±0.142
2	200	84.111±0.046	71.653±0.142	97.741±0.261
3	300	90.214±0.093	80.444±0.284	98.157±0.142
4	400	96.179±3.228	81.097±0.234	99.472±0.026

Values are given as Mean ± SD of three replicate

The significant antioxidant activity of the methanol extract compared to the water extract is attributed to the higher solubility of phenolic compounds and flavonoids in methanol. These phytochemicals are well-known for their ability to donate hydrogen atoms or electrons, neutralizing free radicals like DPPH (Rufino *et al.,* 2011). Phenolics and flavonoids in methanol extracts are more efficient radical scavengers than other compounds, such as polysaccharides, which are more prevalent in aqueous extracts (Wong *et al.,* 2014).

Antioxidant Potential (IC_{50}) of *Nerium indicum*

The IC_{50} values (the concentration required to inhibit 50% of DPPH radicals) for the methanol and water extracts are presented in Table 4. The methanol extract showed an IC_{50} value of 30.218±2.957 µg/mL, while the water extract had a slightly higher IC_{50} of 33.209±0.487 µg/mL, indicating that the methanol extract has better antioxidant potential. Ascorbic acid, used as a reference, had an IC_{50} value of 1.131±0.091 µg/mL, which reflects its superior radical scavenging capacity.

Table 4: Antioxidant Potential (IC_{50}) of Leaf of Extract of *Nerium indicum*

S/No.	Extracts/Standard	Methanol extracts	Water extracts
1	Leaves extracts	30.218±2.957	33.209±0.487
2	Ascorbic acid (std)	1.131±0.091	1.131±0.091

Values are given as Mean ± SD of three replicates

The lower IC_{50} value of the methanol extract compared to the water extract is consistent with other studies showing that methanol efficiently extracts phenolic compounds with potent antioxidant properties. Phenolics, particularly flavonoids and tannins, contribute significantly to antioxidant activity by scavenging free radicals and chelating metal ions, preventing oxidative damage (Rice-Evans *et al.,* 1997). This finding aligns with research on other medicinal plants, such as *Ocimum sanctum* and *Moringa oleifera*, where methanol extracts also demonstrated more substantial antioxidant potential than aqueous extracts (Kumar *et al.,* 2012).

The results indicate that *Nerium indicum* leaf extracts possess significant antioxidant activity, especially in methanol extracts. This is likely due to the higher solubility of phenolics, flavonoids, and tannins in methanol. These compounds are critical in neutralizing free radicals, which are implicated in various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Lobo *et al.,* 2010). The radical solid scavenging capacity of the methanol extract suggests that *Nerium indicum* may be a valuable source of natural antioxidants, potentially helpful in developing nutraceuticals or pharmaceuticals to prevent oxidative stress-related diseases.

The antioxidant activity of *Nerium indicum* observed in this study is consistent with previous reports. Chaudhary *et al.* (2004) found that *Nerium indicum* contains a high concentration of phenolic compounds, contributing to its significant antioxidant properties. Similarly, Sharma *et al.* (2010) reported that the presence of flavonoids and tannins in the leaves of *Nerium indicum* plays a significant role in its antioxidant efficacy. The current study's findings also align with those of Dey *et al.* (2012), who demonstrated that the hydroethanolic extracts of *Nerium indicum* possess strong free radical scavenging capabilities.

The phytochemical composition of *Nerium indicum* is rich in bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and cardiac glycosides, as evidenced by the vigorous DPPH radical scavenging activity observed in both methanol and water extracts. Flavonoids and phenols, in particular, are known for their potent antioxidant properties (Pietta, 2000). The high concentration of these compounds in the methanol extract explains its superior antioxidant activity. The presence of cardiac glycosides and saponins, which also have antioxidant properties, further enhances the therapeutic potential of *Nerium indicum* (Ajinkya *et al.,* 2013).

The antioxidant potential of *Nerium indicum* corroborates its traditional uses in ethnomedicine. The plant has been widely used to treat conditions such as inflammation, cancer, and cardiovascular diseases, all associated with oxidative stress (Dey *et al.,* 2014). The presence of potent antioxidant compounds in the leaf extracts supports these traditional applications and highlights the plant's potential for further development as a therapeutic agent.

CONCLUSION

The phytochemical analysis and antioxidant evaluation of *Nerium indicum* leaf extracts demonstrate that this plant is a valuable source of bioactive compounds with significant therapeutic potential. Methanol proved to be a more effective solvent than water in extracting compounds such as alkaloids, flavonoids, phenols, and cardiac glycosides, known for their diverse pharmacological properties. The methanol extract exhibited more potent antioxidant activity than the water extract, as evidenced by its higher DPPH radical scavenging capacity and lower IC_{50} value. These findings are consistent with previous research on *Nerium indicum* and related medicinal plants, reinforcing the plant's traditional use for treating conditions linked to oxidative stress, such as inflammation, cardiovascular diseases, and cancer. The significant antioxidant activity observed in the methanol extract highlights the potential of *Nerium indicum* as a natural source of antioxidants, making it a promising candidate for developing therapeutic agents against oxidative stress-related conditions. Further research should focus on isolating and characterizing the specific bioactive compounds responsible for the observed antioxidant effects. Additionally, in vivo studies and clinical trials are necessary to validate the therapeutic efficacy of *Nerium indicum* and its potential applications in modern medicine. The continued exploration of this plant's bioactive properties underscores its relevance in the search for natural, plant-based remedies for human health.

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