International Journal of Science and Engineering Science Research
Volume 1 | Issue 1 | January-March 2025 | ISSN **3049-2793**

Comprehensive Analysis of Phytochemical Composition and Antioxidant Activity of *Ventilago Madraspatana* Leaf Extracts

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Abstract

Plants have tremendous medicinal value and have been associated with pharmacologically active phytochemicals for thousands of years. These bioactive compounds, especially phenolics and flavonoids, present strong antioxidant activity which can reduce oxidative stress. However, oxidative stress is known to be an upstream cause in the pathogenesis of many chronic diseases such as cancer, cardiovascular disease, and neurodegenerative disorders. The present study was focused on the investigation of antioxidant qualities by quantifying total phenolic, and flavonoid contents, and evaluating different solvent extracts such as methanol, hexane, and ethyl acetate leaf extract of *Ventilago madraspatana* in terms of free radical scavenging potential.The methanol extract also offers the highest antioxidant activity in HRS and ABTS radical scavenging total antioxidant activity measurements. High antioxidant activity was also found to be well related with high concentrations of phenolic and flavonoid compounds, hence these two groups of compounds are the main responsible for the antioxidant properties of this plant. As expected, the hexane and ethyl acetate extracts exhibited relatively low antioxidant activities but may have interesting perspectives in terms of bioactive compounds including saponins, triterpenes, and phlobaphenes.Consequently, *V. madraspatana* methanol extract was able to show a better amount of antioxidant activity with the previous findings that reveal the potential of plants as natural antioxidantsources and support their use in folk medicine only for curative purposes. In addition, the findings will be used as a basis for future studies to identify and characterize key compounds that are known to possess therapeutic properties with an emphasis on preventing or treating diseases associated.

Keywords: *Ventilago madraspatana*, Phytochemical screening, Quantitative, Antioxidant, HRS, ABTS

1. Introduction

1.1 Background of Medicinal Plants

For centuries, medicinal plants have been key components of the traditional healthcare systems of many cultures and used for both the prevention and management of numerous diseases. Secondary metabolites like alkaloids, flavonoids, tannins, phenolics, and glycosides that provide various pharmacological actions are often present in them. In particular plant derived antioxidants are gaining considerable attention due to their capacity to counteract free radicals and reduce oxidative stress in biological systems.

Oxidative stress is due to an increased imbalance between the generation of reactive oxygen species (ROS), particularly peroxide, and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. ROS are reactive molecules capable of causing damage to proteins, lipids, and DNA which in turn contributes to the pathogenesis of chronic diseases. This is counteracted by antioxidants, which are scavengers of free radicals that scavenge the damage to cells.

1.2 Significance of *Ventilago Madraspatana*

It is a common plant in tropical regions particularly in Asia and Africa. This species has been traditionally used for various health conditions including inflammatory maladies, burns, and gastrointestinal disorders. Despite being widely used there are scanty scientific data on the phytochemical composition and antioxidant potential of this plant.

The phytochemical composition and antioxidant activity of *V. madraspatana* leaf extracts obtained through different solvents were determined to identify and quantify bioactive compounds (phenolics and flavonoids) for a better understanding of the medicinal value of V. madraspatana and its potential application in healthcare.

1.3 Antioxidants and their Role in Human Health

Flavonoids are distinguished by their ability to scavenge free radicals, decrease inflammation, and have a role in preventing several degenerative diseases. On the other hand, Phenolic compounds are known for their antioxidant activities that protect cells from oxidative damage by donating hydrogen atoms or electrons to neutralize free radicals.

Given the potential health benefits associated with these compounds, there is an increasing interest in finding plant-based sources of these compounds, and *V. madraspatana*, being rich with phytochemicals based on its traditional medicinal uses, suits this purpose.

2. Materials and Methods

2.1 Collection of Plant Material

Leaves of *Ventilago madraspatana* were collected from its natural habitat (tropical region). The plant material was authenticated by a qualified botanist. Freshly collected leaves were washed thoroughly with

distilled water to remove dust, dirt and other contaminants and subsequent- ly air-dried in the laboratory at room temperature for several days until a constant weight was obtained. Air-dried leaves were then pulverized to a fine powder using a mechanical grinder and stored until required for study[1].

2.2 Preparation of Extracts

Methanolic, hexane, and ethyl acetate leaf extracts were further prepared from powdered leaf material. Solvent extraction was performed based on relative solvent polarity, with methanol being highly polar, hexane neutral and ethyl acetate being moderately polar.

- **Methanol Extraction**: The methanolic extract was prepared by soxhlet apparatus where about 50 gm of powdered leaves were extracted at a time with 6-8 hour repeated extraction with the help of solvent methanol [2]. The extract obtained was filtered with the help of Whatmann filter paper to remove the solid residue. Then it is concentrated using a rotary evaporator under low temperatures to obtain the crude extract methanol extract.
- **Hexane Extraction**: Hexane extraction was also done by using a soxhlet apparatus under similar conditions to methanol extraction to get hexane extract.
- **Ethyl Acetate Extraction** Ethyl acetate extraction was performed by the Soxhlet method to extract compounds of intermediate polarity.

Every one of the generated crude extracts was placed in sealed containers at 4°C to maintain their integrity until additional analysis for phytochemical screening and antioxidant activity could be carried out [3].

2.3 Phytochemical Screening

Phytochemical screening aimed to find secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics in the extracts. Standard qualitative tests were employed as follows:

- **Alkaloid Test:** Using Dragendorff's reagent, we established that a precipitate of orange color appeared when mixed with the extract in the presence of alkaloids.
- **Flavonoid Test**: Detected using the Shinoda test were [4] In this procedure, the extract incorporated magnesium turnings and concentrated hydrochloric acid. A conversion to pink or red showed the existence of flavonoids.
- **Phenolic Compound Test:** To test for phenolic compounds, the Folin-Ciocalteu reagent was brought into use. Incorporating sodium carbonate into the extract caused a blue color change which confirmed that phenolics were present.
- **Saponins Test:** The purpose of the froth test was to identify saponins. A durable foamy layer that developed after shaking the extract with water revealed their existence [5].

2.4 Quantitative Analysis

2.4.1 Total Phenolic Content

The quantity of total phenolic content in the extracts was measured using the Folin-Ciocalteu reagent procedure. The use of gallic acid as a standard resulted in the expression of results in milligrams of gallic acid equivalents (GAE) per gram of extract [6]. The technique comprised combining a recognized volume of the plant extract with the Folin-Ciocalteu reagent before adding sodium carbonate solution. The mixture stayed incubated for thirty minutes at ambient conditions before the absorbance measurement at 760 nm using a spectrophotometer [7].

2.4.2 Total Flavonoid Content

We determined total flavonoid content using the aluminum chloride colorimetric method, in which quercetin functioned as the reference standard. The findings were shown in milligrams of quercetin equivalents (QE) per gram of extract. The extract was added to aluminum chloride and potassium acetate and kept at incubation for 30 minutes. The measure of absorbance for the produced mixture was taken at 415 nm[8].

2.5 Antioxidant Activity Assays

2.5.1 Hydroxyl Radical Scavenging (HRS) Assay

The activity against hydroxyl radicals of the extracts was investigated using the HRS assay. The Fenton reaction produced hydroxyl radicals, and the measuring of extract inhibition of deoxyribose degradation followed [9]. The combination included FeSO4, EDTA, deoxyribose, and H2O2, along with the plant extract. After 1 hour at 37°C, the absorbance measurement took place at 532 nm. The percentage of hydroxyl radical scavenging was calculated using the formula:

$$\text{HRS Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

- A_{control} is the absorbance of the control (without extract)
- A_{sample} is the absorbance of the sample with the plant extract

The equation expresses the percentage of hydroxyl radical inhibition, measuring the capability of the extract to neutralize them [10].

2.5.2 ABTS Radical Scavenging Assay

The ABTS assay was conducted to evaluate the ability of the extracts to scavenge ABTS radicals. ABTS radical cation was generated by mixing ABTS stock solution with potassium persulfate and allowing the reaction to proceed in the dark for 24 hours [11]. The extract was then added to the ABTS solution, and the absorbance was measured at 734 nm. The percentage inhibition of ABTS radicals was calculated similarly to

the HRS activity, with IC50 values also determined for comparative analysis among the different extracts [12].

For the ABTS assay, the percentage inhibition of the ABTS radical cation is calculated using a similar equation:

$$\text{ABTS Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

- A_{control} is the absorbance of the control (without extract)
- A_{sample} is the absorbance of the sample with the plant extract

This calculation reflects the percentage of ABTS radical cation scavenged as a result of the extract [13].

2.6 Statistical Analysis

All assays performed were in triplicate to verify reproducibility, and results were shown as mean ± standard deviation (SD). We carried out statistical analysis using one-way ANOVA followed by Tukey's post hoc test to evaluate whether there are significant differences between the antioxidant activities of the differing extracts. We deemed a p-value less than 0.05 to be of statistical significance[14].

3. Results

3.1 Phytochemical Screening

Screening for phytochemicals in the leaves of *Ventilago madraspatana* revealed a variety of active compounds, including alkaloids, flavonoids, phenolics, saponins, and tannins, in their methanol, ethyl acetate, and hexane extracts [15]. The methanol extract exhibited, as shown in Table 1, the greatest concentrations of flavonoids, phenolics, and alkaloids. This is important due to the known crucial functions of these secondary metabolites in the antioxidant capacities of plant extracts. Due to their properties, flaxonoids and phenolic compounds, primarily, exhibit potent free radical scavenging and can help to alleviate oxidative stress. Rather, according to the findings, the extract prepared with hexane demonstrated the least amount of these metabolites, suggesting that solvents like methanol are better at extracting bioactive substances from leaves of *V. madraspatana*[16].

Table 1: Phytochemical Composition of Ventilago Madraspatana Leaf Extracts

Test	Hexane	Ethyl Acetate	Methanol

Alkaloids	++	++	+++
Flavonoids	++	++	+++
Tannins	+	+	+++
Coumarin	+	+	++
Saponin	-	-	-
Glycosides	-	-	+
Terpenoids	-	-	+
Phenolics	++	++	+++

(+ Trace amount, ++ Moderately present, +++ Highly present, - Absent)

Table 1 outlines the results of the phytochemical screening of Ventilago madraspatana leaf extracts obtained using three different solvents: Methanol, ethyl acetate, and hexane are all parts of it. The table groups the incidence of multiple secondary metabolites, all of which include alkaloids, flavonoids, tannins, coumarin, saponins, glycosides, terpenoids, and phenolics. The findings show that the methanol extract contains a markedly higher level of most phytochemicals, particularly flavonoids, phenolics, and alkaloids, which are all considered highly present (+++). Methanol's effectiveness in extracting these bioactive compounds, which are necessary for antioxidant activity, is suggested by this. In opposition, the hexane extract reflects the smallest amount of these metabolites, supporting the common thought that non-polar solvents are less capable of extracting polar compounds. This result stresses the importance of the solvent in establishing the phytochemical profile available from plant materials[17].

3.2 Total Phenolic and Flavonoid Content

An analysis of total phenolic and flavonoid levels in the methanol extract showed it contained the highest concentrations followed by the ethyl acetate extract, whereas the hexane extract showed the smallest amounts. The strong antioxidant effects of phenolic compounds and flavonoids greatly contribute to the total antioxidant power of plant extracts. The total phenolic concentration in the methanol extract was 120.15 mg GAE/g, together with a total flavonoid content of 98.76 mg QE/g. The findings conform to past research that indicates methanol is an adequate solvent for the extraction of polar phenolic and flavonoid compounds[18].

The phenolic total is computed as gallic acid equivalents (GAE) following a standard curve for gallic acid. The formula is:

$$\text{Total Phenolic Content (mg GAE/g extract)} = C \times \frac{V}{M}$$

Where:

- C is the concentration of phenolics obtained from the calibration curve (mg/mL)
- V is the volume of the extract (mL)
- M is the mass of the extract (g)

The presented Equation calculates the total phenolic content in milligrams of gallic acid equivalents for each gram of extract[19].

We determine the total flavonoid content using a standard curve created with quercetin equivalents (QE). The equation is:

$$\text{Total Flavonoid Content (mg QE/g extract)} = C \times \frac{V}{M}$$

Where:

- C is the concentration of flavonoids obtained from the calibration curve (mg/mL)
- V is the volume of the extract (mL)
- M is the mass of the extract (g)

This equation gives the flavonoid content relative to milligrams of quercetin equivalents for each gram of plant extract.

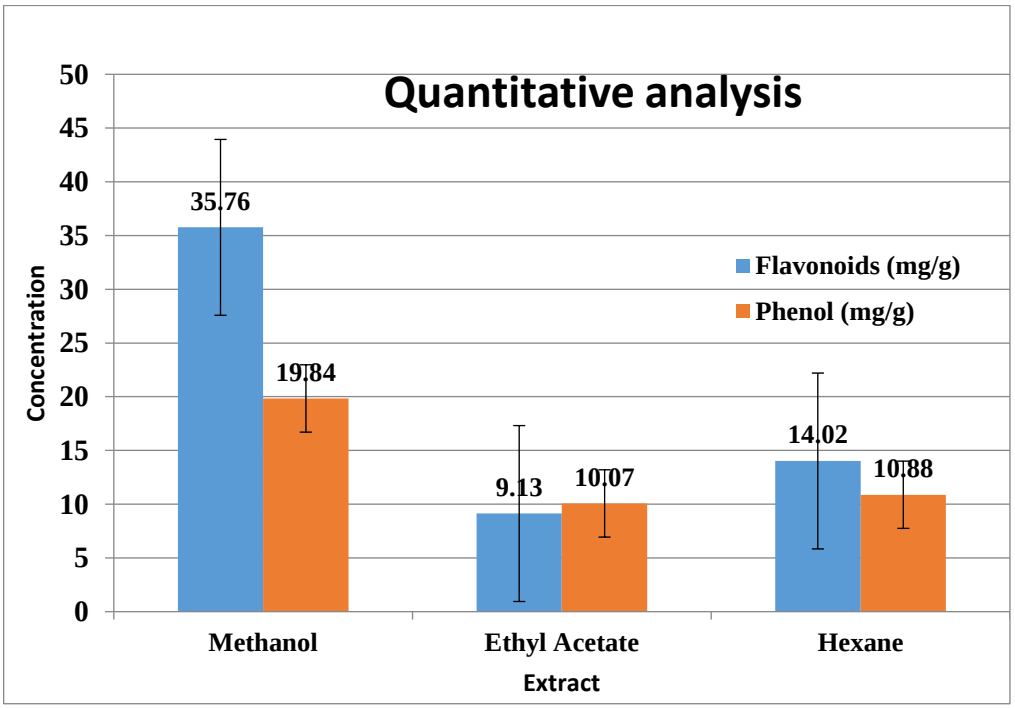
Table 2: Total Phenolic and Flavonoid Content of *Ventilago Madraspatana* Leaf Extracts

Bioactive compounds	Extraction content		
	Methanol	Ethyl acetate	Hexane
Total Polyphenols (GAE[1] mg/g)	19.84±0.26[a]	10.07±0.17[c]	10.88±0.15[b]
Total Flavonoids (QE[2] mg/g)	35.76±0.67[a]	9.13±0.18[c]	14.02±0.52[b]

Quantitative measurements of the phenolic and flavonoid contents in the three extracts are shown in Table 2, expressed in Gallic Acid Equivalents (GAE) and Quercetin Equivalents (QE) per gram. The results illustrate that the methanol extract contains the maximal levels of both total phenolics (19.84 ± 0.26 mg GAE/g) and total flavonoids (35.76 ± 0.67 mg QE/g). The ethyl acetate extract gives off moderate levels (10.07 ± 0.17 mg GAE/g and 9.13 ± 0.18 mg QE/g), in contrast to the hexane extract, which records the least amounts of these compounds (10.88 ± 0.15 mg GAE/g and 14.02 ± 0.52 mg QE/g). The data corresponds with earlier

findings from the phytochemical screening, more strongly confirming that methanol is a more competent solvent for extracting phenolic and flavonoid compounds, which are important to the antioxidant potential of the extracts[20].

Figure-1: Quantitative analysis of phytoconstituents from various solvent leaf crude extracts of *Ventilago madraspatana* Gaertn.



3.3 Antioxidant Activity

The antioxidant activities of the methanol, ethyl acetate, and hexane extracts were determined using Hydroxyl Radical Scavenging (HRS) assays along with ABTS radical scavenging assays[21]. Data shown in Tables 3 and 4 indicate that the methanol extract presented the highest level of scavenging activity in each assay. At concentrations of 200 µg/ml, the methanol extract exhibited the greatest inhibition,which reached 83.65% and 79.02% in the HRS and ABTS assays respectively.The hexane extract, in opposition, proved to have the least scavenging activity, with inhibition rates of 60.27% and 50.65% at 200 µg/ml for the HRS and

ABTS assays respectively. Findings confirm that methanol, as a polar solvent, achieves better extractions of antioxidants than hexane, a non-polar solvent.The methanol extract produced IC50 values that were markedly inferior to those of the ethyl acetate and hexane extracts, thus emphasizing its superior antioxidant properties[22].

Table 3: Hydroxyl Radical Scavenging (HRS) Activity of *Ventilago Madraspatana* Leaf Extracts

Concentration (µg/ml)	Extraction content		
	Methanol	Ethyl acetate	Hexane
20	42.96±0.68^{a}	31.01±0.76^{b}	19.97±0.15^{c}
40	46.04±0.66^{a}	34.05±0.69^{b}	23.24±0.71^{c}
60	52.18±0.81^{a}	37.08±0.72^{b}	26.74±0.56^{c}
80	54.02±0.61^{a}	42.83±0.53^{b}	30.96±0.63^{c}
100	60.88±0.48^{a}	46.94±0.61^{b}	35.08±0.16^{c}
120	64.04±0.70^{a}	53.08±0.82^{b}	38.88±0.75^{c}
140	67.17±0.48^{a}	56.12±0.80^{b}	43.27±0.38^{c}
160	72.23±0.27^{a}	62.41±0.64^{b}	47.19±0.82^{c}
180	77.18±0.93^{a}	68.22±0.57^{b}	50.11±0.75^{c}
200	80.90±0.69^{a}	71.19±0.42^{b}	53.22±0.34^{c}

The average of triplicate is where values find their expression, alongside mean ± SD. The Methanol, Ethyl Acetate and Hexane extracts (n=3) differ with a $P<0.05$ on their comparison (n=3).

Table 3 reports the Hydroxyl Radical Scavenging (HRS) activity over a range of concentrations (20 to 200 µg/ml) for the leaf extracts. At all tested concentrations, the methanol extract constantly reveals the highest percentage of radical scavenging activity, reaching an impressive 80.90 ± 0.69% inhibition at 200 µg/ml. On the other hand, the hexane extract reveals markedly diminished scavenging ability, reporting the greatest inhibition at just 53.22 ± 0.34%. The increasing rate of scavenging activity concentrating on all extracts points to V. madraspatana's promise as a source of antioxidants, notably with the methanol extract showing superior performance. The statistical markers ($P < 0.05$) seen in the outcomes show that the variations in antioxidant capacity across the extracts are statistically important, underpinning the thesis that methanol is the optimal solvent for acquiring antioxidant compounds[23].

Figure: 2 Hydroxyl radical scavenging activity of various solvent leaves Extracts of *Ventilago madraspatna* Gaertn.

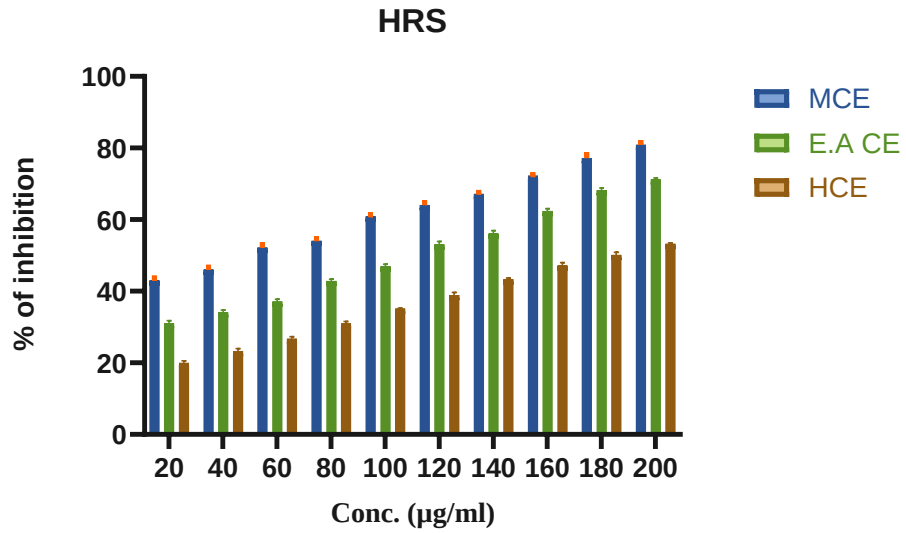


Table 4: ABTS Radical Scavenging Activity of *Ventilago Madraspatana* Leaf Extracts

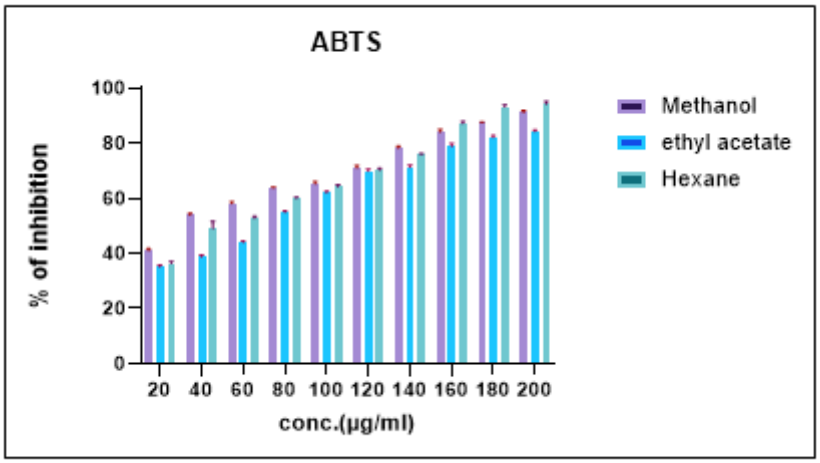
Concentration (µg/ml)	Methanol (%)	Ethyl Acetate (%)	Hexane (%)
20	48.79 ± 0.45	27.21 ± 0.51	21.15 ± 0.38
40	52.03 ± 0.33	30.18 ± 0.61	24.13 ± 0.29
200	79.02 ± 0.62	70.47 ± 0.77	50.65 ± 0.72

The average of triplicate along with mean ± SD represents values. P>0.05 for evaluation of the Methanol, the Ethyl Acetate, and the Hexane extracts (n=3)

As shown in Table 4, the leaf extracts underwent a radical scavenging assay at concentrations of 20, 40, and 200 µg/ml. The methanol extract indicates the largest radical scavenging activity once more, revealing a maximum of 79.02 ± 0.62% inhibition at 200 µg/ml. With 70.47 ± 0.77% inhibition present, the ethyl acetate extract shows intermediate action, and the hexane extract is the least effective, presenting 50.65 ± 0.72% inhibition at a concentration of 200 µg/ml[24]. The results conform to those seen in the HRS assay and suggest that the methanol extract is a rich source of antioxidants. Statistical analysis (P > 0.05) shows

that the differences in scavenging activity between the various extracts are important; thereby affirming the effect of solvent choice on the antioxidant potential of plant extracts [25].

Figure 3: ABTS Radical scavenging activity of various solvent leaves Extracts of *Ventilago madraspatana* Gaertn.



The extract of Ventilago madraspatana leaves in methanol showed the greatest antioxidant activity according to results from both the HRS and ABTS methods. This is associated directly with its higher phenolic and flavonoid concentrations, which serve an important function in free radical scavenging[26]. Notably, the ethyl acetate extract showed moderate antioxidant activity, in contrast, the hexane extract showed meek antioxidant potential. The results emphasize the role of solvent choice in obtaining bioactive compounds from the plant material and propose that extracts from V. madraspatana leave particularly the methanol extract, which maybe a powerful source of natural antioxidants for use in pharmaceuticals or nutraceuticals[27].

4. Discussion

This study shows that the methanol extract of Ventilago madraspatana leaves presents the greatest total antioxidant capacity and reducing power concerning the hexane and ethyl acetate extracts. The distinguishing feature is essentially due to the amplified amounts of phenolic and flavonoid compounds found in the methanol extract. A variety of studies have established the key importance of these compounds for antioxidant processes. As an example, the capacity of flavonoids to scavenge oxidizing agents such as singlet oxygen and assorted free radicals is widely acknowledged. Crucial to alleviating oxidative stress, a chief factor in the onset of a variety of chronic diseases, is this ability to scavenge[28].

Our findings suggest that the methanol extract had both better scavenging activity in the Hydroxyl Radical Scavenging (HRS) and ABTS assays and produced significantly lower IC50 values when compared to the hexane and ethyl acetate extracts. The IC50 value measures the level of concentration required to affect 50% of free radicals, and smaller values indicate greater antioxidant strength[29]. The findings support the argument that methanol is an especially powerful solvent for the extraction of bioactive ingredients from V. madraspatana leaves, stressing the impact of solvent choice in phytochemical extraction. Previously published work has indicated that methanol extracts obtained from a range of plant species repeatedly show superior antioxidant activity when compared to those derived using non-polar solvents.

Although possessing some antioxidant properties, the hexane and ethyl acetate extracts were markedly less effective than their counterpart methanol extract. We can understand this difference by analyzing the polarity variations of the solvents. Polar solvents, particularly methanol, are better able to dissolve polar compounds such as phenolics and flavonoids, important for functions including antioxidation. Alternatively, non-polar solvents, such as hexane, are not as effective at extracting these compounds and hence yield lower antioxidant potential[30].Ultimately, the findings of the study prove how important V. madraspatana leaves are as a potential natural antioxidant source, especially in terms of methanol extraction. Not only does the methanol extract validate traditional herbal medicine uses of this plant, but it also suggests its usefulness for pharmaceutical and nutraceutical purposes. Research in the future must concentrate on finding particular bioactive compounds from the methanol extract and examining their restorative properties, especially in diseases associated with oxidative stress.

5. Conclusion

In a nutshell, this research points out that Ventilago madraspatana leaves contain a range of bioactive compounds, with phenolics and flavonoids being particularly key to the plant's antioxidant function. Analyses of the extracts indicated that the methanol extract showed the greatest antioxidant activity, implying its potentially beneficial therapeutic applications, particularly in handling diseases related to oxidative stress.These results lay the groundwork for future investigations focused on isolating and characterizing specific bioactive substances from V. madraspatana, as well as exploring their therapeutic potential, especially in cancer therapies. Moreover, expanded research into the mechanisms that govern the antioxidant activity of the methanol extract could give a valuable understanding of its medicinal characteristics and enable its use in pharmaceutical and nutraceutical applications.

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