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Phytochemical, Antimicrobial, *In Vitro* Antioxidant and Anti-Inflammatory Activity Studies of *Artemisia pallens*

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ABSTRACT

This study investigates the phytochemical constituents, antimicrobial characteristics, and the antioxidant and anti-inflammatory activities of methanolic leaf extract of *Artemisia pallens*. The screening revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids. Utilizing the agar well diffusion method, an inhibitory effect of the essential oil on bacterial growth was observed. Various in vitro assays were conducted to evaluate the antioxidant activity of the extract. Additionally, the anti-inflammatory properties of the aerial parts were also examined.

Keywords: *Artemisia pallens,* Asteraceae, Phytochemical Screening, Antioxidant Activity, DPPH

INTRODUCTION

Medicinal plants have been utilized for centuries to treat human diseases, thanks to their valuable therapeutic components. In recent years, interest in naturally occurring antioxidants has flourished, especially for applications in food, cosmetics, and pharmaceuticals [1].

This growing fascination is due to the diverse activities these compounds offer and their ability to help correct physiological imbalances. Research has demonstrated a strong correlation between the antioxidant potential of certain fruits and vegetables and their total phenolic content. The effectiveness of phenolic compounds as antioxidants primarily arises from their redox properties, which enable them to absorb and neutralize free radicals, as well as to quench singlet and triplet oxygen and decompose peroxides [2].

Plants within the genus Artemisia are predominantly small, fragrant shrubs or herbs, many of which yield essential oils. These oils can serve various medicinal purposes, such as acting as vermifuges or stimulants, and they are also employed in perfumery. Furthermore, the leaves of some Artemisia species are valued as culinary herbs, and these plants are favored by gardeners for their ornamental qualities [3].

Artemisia pallens, commonly referred to as “Davana” in Ayurvedic medicine and belonging to the Asteraceae family, stands out as a versatile medicinal plant. It is frequently used on its own or in combination with other herbs to address a wide range of health issues. The leaves and flowers of *Artemisia pallens* are notable for producing an essential oil known as ‘oil of Davana’ [4]. Given its renowned fragrance and biological activities, there is a compelling case for conducting further studies on the phytochemical properties, biological effects, and antioxidant activities of the aerial parts of *A. pallens*, which could uncover valuable insights and applications in various fields.

MATERIALS AND METHODS

Collection, extraction and preparation of the plant material

Fresh aerial parts of *Artemisia pallens* were sourced from a local market in Thiruvananthapuram and subsequently identified by a botanist. The plant material was chopped, dried, and then finely powdered. Fifty grams of this powdered material were subjected to Soxhlet extraction using methanol. The extracts were collected, and the solvent was removed using a rotary evaporator under reduced pressure to yield the crude extracts. These extracts were then appropriately diluted with methanol to prepare various concentrations for phytochemical screening, antioxidant activity assessment, and anti-inflammatory activity studies.

Phytochemical screening

Multiple colorimetric reactions had been employed for the preliminary identification of various phytochemicals present in the methanol extract of *A. pallens* [5] [6] [7] [8] [9].

Antimicrobial assay

In the study of antimicrobial activity, essential oil was extracted through hydrodistillation using a Clevenger apparatus. For this purpose, 50 g of the aerial parts of *Artemisia pallens* were collected, which were then dried and powdered before the extraction process. The resulting essential oil was subjected to screening against a range of bacterial and fungal species to evaluate its antimicrobial properties.

Antioxidant and anti-inflammatory activity studies

The extract was subjected to a series of *in vitro* assays to evaluate its antioxidant activity and anti-inflammatory properties, following established procedures documented in scientific literature.

RESULTS AND DISCUSSION

Phytochemical screening

The results from the color tests clearly demonstrate that the methanol extract of the aerial parts of *A. pallens* is rich in a diverse array of phytochemicals. The presence of a particular phytochemical is represented by the symbol (+) and its absence is represented by the symbol (⁻).

Table 1. Results of Preliminary Phytochemical Screening Tests

Sl. No.	Test for various phytochemicals	*A. pallens extract*
1	Carbohydrates	-
2	Cardiac glycosides	+
3	Terpenoids	+
4	Alkaloids	+
5	Flavonoids	+
6	Quinones	+
7	Tannins	+
8	Resins	+
9	Anthraquinones	-
10	Coumarins	+
11	Phytosterols	+
12	Gums and mucilages	+
13	Oxalates	+
14	Betacyanins	-

Antimicrobial Assay

The essential oil derived from *Artemisia pallens* was assessed for its *in vitro* antimicrobial activity against a selection of bacterial and fungal species using the agar well diffusion method [10]. The investigation focused on several bacterial species: (1) *Staphylococcus aureus* and (2) *Streptococcus mutans*, both classified as gram-positive, as well as (3) *Escherichia coli* and (4) *Pseudomonas aeruginosa*, which are gram-negative. Furthermore, the study also encompassed the fungal species *Candida albicans* and *Aspergillus niger*. This comprehensive approach highlights the potential of *Artemisia pallens* as a natural antimicrobial agent worth exploring further.

Table 2. Results of Antimicrobial Assays

Organisms	Gram +ve/-ve	Diameter of the zone of inhibition (mm)	
		Essential oil of Aerial parts	Standard
Staphylococcus aureus	+	1.5	3.0
Streptococcus mutans	+	1.7	2.7
Escherichia coli	-	1.0	3.2
Pseudomonas aeruginosa	-	1.2	2.8
Aspergillus niger	Fungal	Nil	1.9
Candida albicans	Fungal	Nil	1.5

The growth of microorganisms on the plate was clearly observed (Figure 1). The diameter of the zone surrounding the disc where the essential oil was applied effectively indicates the extent of microbial deactivation, which reflects the antimicrobial potency of the essential oil against the specific microorganism. A larger diameter directly demonstrates a superior ability to inhibit growth, affirming the remarkable potential of *Artemisia pallens* as a powerful source of natural antimicrobial agents. This finding strongly supports further investigation into its applications in microbiology and natural product development.

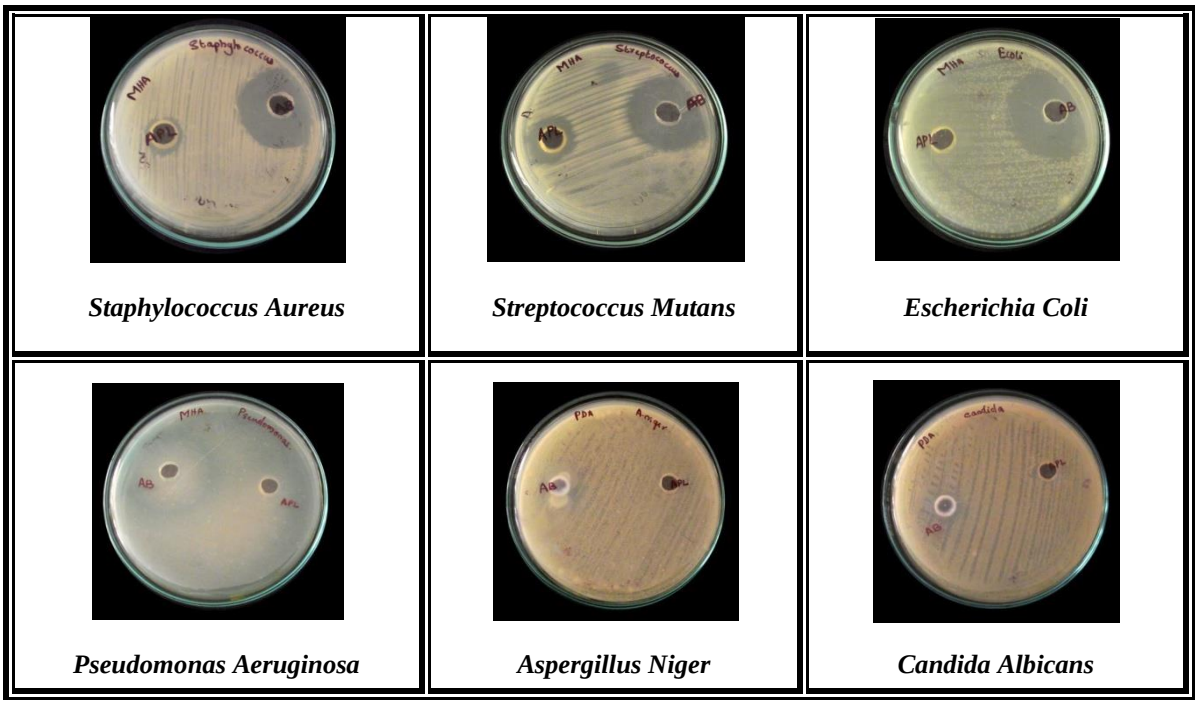

Figure 1: Growth of micro organisms

From the experiment, it was observed that, among the six micro organisms tested, four of them were deactivated by the essential oil. They were *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* and *Pseudomonas aeruginosa*. However, it is noteworthy that the essential oil of Artemisia pallens did not exhibit any activity against the two fungal species assessed.

ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY STUDIES

Antioxidant activity by DPPH$^{\bullet}$ assay

The antioxidant activity of *A. pallens* was evaluated using its methanol extract. Various concentrations of the extract were prepared and treated with DPPH$^{\bullet}$, following the established methodology for absorbance measurement [11]. The percentage of radical scavenging capacity was subsequently calculated. The findings are illustrated in Figure 2 and summarized in Table 2.

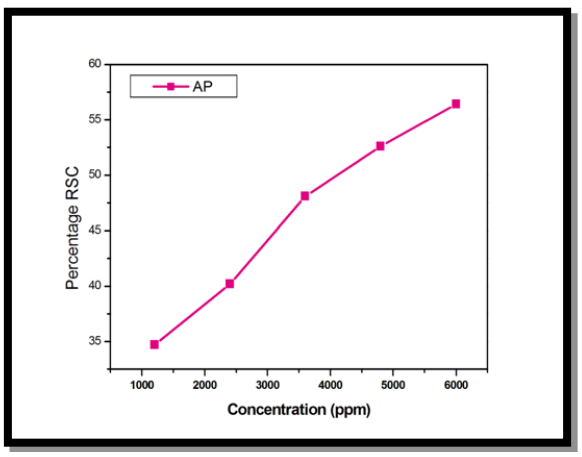

Figure 2. Percentage scavenging of DPPH$^{\bullet}$ radical at different concentrations of *A. pallens* extract

The EC_{50} value of *A. pallens* is found to be 4109.55 $\pm$ 2.51 ppm.

Estimation of total phenolic content

In this analysis, 1000 ppm of each of the extracts were treated with F-C reagent and sodium carbonate, following the standard procedures outlined in the literature [12]. This allowed to accurately measure the absorbance. Notably, it is found that the total phenolic content of *A. pallens* extract was 1.57 $\pm$ 0.24 g GAE per 100 g of extract, providing valuable insight into its composition.

Estimation of total flavonoid content

In the experiment, 2000 ppm of the extract was treated with sodium nitrite, aluminum chloride, and sodium hydroxide, and the absorbance was recorded following the established procedure from the literature [13]. The total flavonoid content found in the *A. pallens* extract was measured to be 3.98 $\pm$ 0.73 g of quercetin equivalents per 100 g of extract.

Table 2. Results of antioxidant and anti inflammatory activity studies

Scavenging of DPPH˙ radical, EC_{50} (ppm)	Total Phenolic content [g GAE / 100 g extract]	Total Flavonoid content [g QE/ 100 g extract]	Superoxide scavenging ability, LD_{50} (□L)	Nitric oxide scavenging ability , LD_{50} (□L)
4109.55 $\pm$ 2.51	1.57 $\pm$ 0.24	3.98 $\pm$ 0.73	726.06 $\pm$ 1.86	2184.48 $\pm$ 3.98

Reducing power

Various concentrations of *A. pallens* extract were treated with potassium ferricyanide, trichloroacetic acid and ferric chloride and then the absorbance was noted as per procedure in the literature [14].

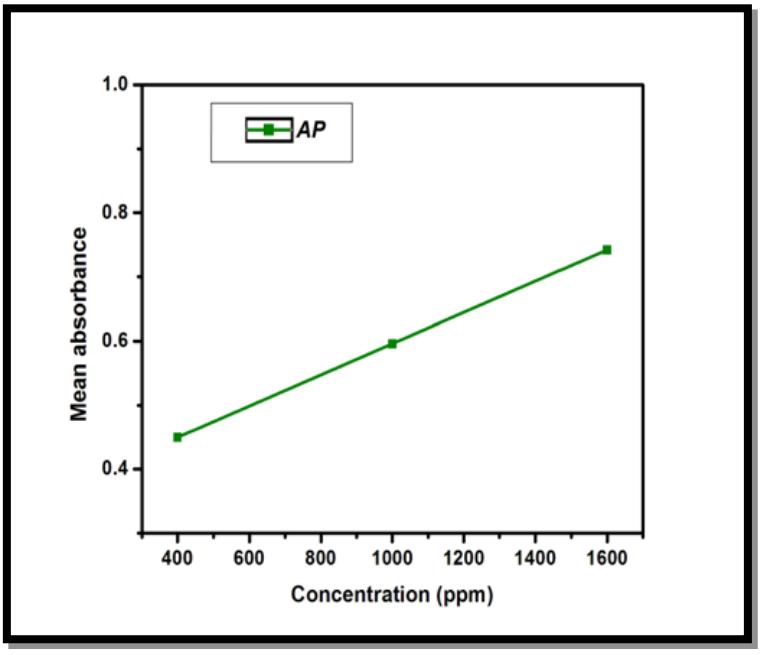

Figure 3. Reducing power at different concentrations of *A. pallens* extract

The data obtained from the experiment revealed that *A. pallens* extract functions as a good reductant. This is observed from the higher absorbance at 700 nm in a dose dependent manner at concentrations of 400 – 1600 ppm.

Superoxide free radical scavenging activity

Measurement of superoxide anion scavenging activity was performed based on the method described by Valentao et al [15]. The percentage inhibition was calculated by comparing the results of control and test samples. The results are illustrated in the following figure 4.

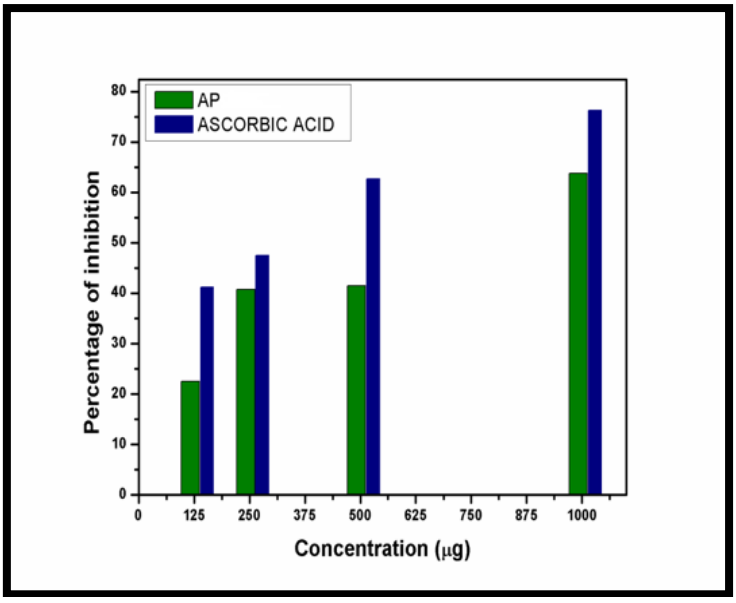

Figure 4. Percentage inhibition of superoxide radical at different concentrations of *A. pallens* extract

A graph was plotted with concentration against percentage of inhibition and, the lethal dose at 50% concentration (LD_{50}) was obtained. The results show that the extract exhibited very good superoxide scavenging ability. The LD_{50} value was found to be 726.06 $\pm$ 1.86 µL.

Nitric oxide scavenging activity

The methanolic extract of the aerial parts of *A. pallens* were used for determining the nitric oxide scavenging ability. Different concentrations of the extract in methanol were treated with sodium nitroprusside in phosphate buffer and Griess reagent as per the standard procedure reported in literature [16]. The absorbance was measured at 546 nm and the percentage scavenging activity was measured with reference to gallic acid which is the standard taken [16].

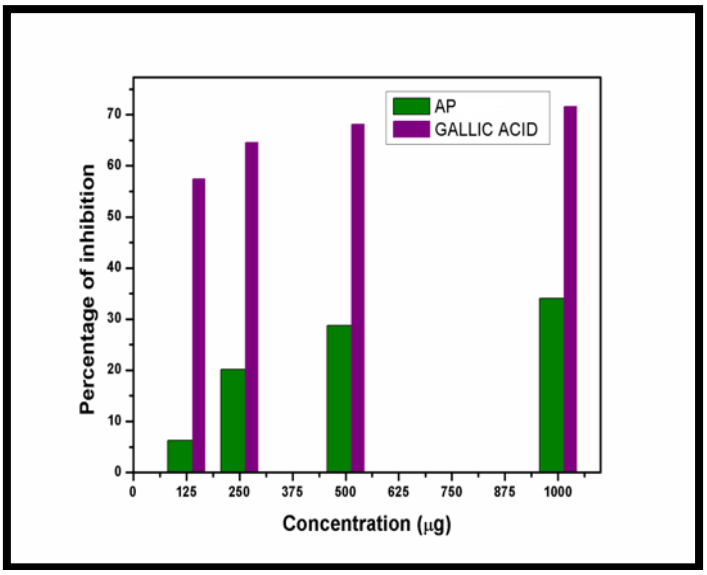

Figure 5. Percentage inhibition of nitric oxide radical at different concentrations of *A. pallens* extract

A graph was plotted with concentration against percentage of inhibition (figure 5). The lethal dose at 50% concentration (LD_{50}) was obtained. The results show that the extract exhibited appreciable nitric oxide scavenging ability. The LD_{50} of the extract is found to be 2184.48 $\pm$ 3.98 µL.

Anti-inflammatory activity

LPS stimulated RAW 264.7 cells were exposed with different concentration (25,50, 100 µg/mL) of extract and Diclofenac sodium, a standard anti-inflammatory drug in varying concentration corresponding to the sample was added and incubated for 24 hours. After incubation the anti-inflammatory assays were performed using the cell lysate. After

completion of the reaction, cyclooxygenase (COX) activity was determined by the method of Walker and Gierse by reading absorbance at 632 nm [17]. The results are presented in the figure 6.

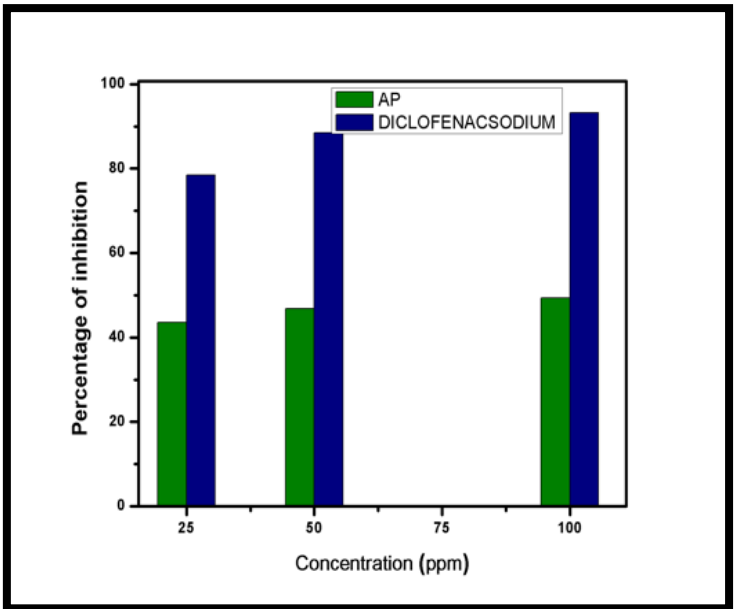

Figure 6. Percentage inhibition (COX activity) at different concentrations of *A. pallens* extract

CONCLUSION

Essential oil of *Artemisia pallens* exhibited good antimicrobial activity when screened against various microorganisms. Methanolic extract of the aerial parts of *Artemisia pallens* was screened for the presence of phytochemicals, antioxidant activity as well as anti-inflammatory activity using various chemical methods. The extract of the aerial parts of *Artemisia pallens* exhibited appreciable antioxidant and anti-inflammatory activity.

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