Phytochemical, antioxidant and a study of bioactive compounds from *Artemisia pallens*

K Sai Pavithra, Jeyanthi Annadurai and R Ragunathan

Abstract

*Artemisia pallens* has been used in Ayurveda for the treatment of measles, cough, cold, depression, diabetes and high blood pressure. The present investigation is focussed to evaluate the antidiabetic activity of *A. pallens* leaf extracts. The water, methanol, chloroform, N-butanol and diethyl ether extracts of my plant was evaluated. The extracts were analysed for DPPH, phenols, FRAP, antioxidants, SOD and hydrogen peroxide. The extracts were evaluated using enzymatic studies, FTIR, GC-MS and the antimicrobial activity. The water, methanol and chloroform extracts appear to show good results than N-butanol and diethyl ether extracts. The antidiabetic activity of *A. pallens* might be due to presence of many natural compounds which might play a role in controlling diabetes.

Keywords: Asteraceae, phytochemicals, quantitative analysis, enzymatic analysis, FTIR, GC-MS, antimicrobial assay

1. Introduction

*Artemisia pallens* Wall. ex Dc is an aromatic herb belonging to Asteraceae family and is native of India and other tropical countries. Artemisia pallens has been used in Ayurveda for the treatment of measles, cough, cold, depression, diabetes and high blood pressure. Traditionally, the leaf powder is used as an antidiabetic, antihypertensive, and antidepressant medicine. The therapeutic potential is due to the presence of natural bioactive compounds. The antidiabetic activity of the plant has not been explored [1]. Diabetes is a metabolic disorder characterized by the rise or fall of the blood sugar levels. The amount of people suffering from diabetes throughout the world is estimated to be 2.8% and is predicted to double every 20 years up to 2040 [2]. Oral intake of high doses of aqueous or methanolic extract of the aerial parts of the plant was shown to decrease blood glucose level in glucose−fed hyperglycemic and alloxan-treated rabbits and rats [3]. Methyl cinnamate, ethyl cinnamate, bicyclogermacrene, 2-hydroxyisodavanone, farnesol, geranyl acetate, sesquiterpene lactones, germacranolides seems to be the important components which are present [4]. The leaves and flowers are of high value in preparing floral decorations and essential oils. The leaves are very small, bluish green with yellow inconspicuous flowers. It is utilized in traditional Ayurvedic medicinal formulations. Essential oil of Artemisia pallens is used as an antiseptic and disinfectant [5]. Thus, the aim of the study is to investigate the antidiabetic activity of *A. pallens* and further chemical characterization of the extracts by phytochemical assays, the quantitative, enzymatic studies, FTIR, GC-MS and the antimicrobial analysis.

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2. Materials and Methods
2.1 Plant collection and extract preparation
The *A. pallens* was collected from Coimbatore, Tamil Nadu, India. The identity of the plant was confirmed by the Botanical Survey of India, Coimbatore, Tamil Nadu, India. The extract was prepared using dried leaves and stem in water, methanol, chloroform, N-butanol and diethyl ether using a shaker. The extracts were stored in sealed containers.

2.2 Qualitative phytochemical analysis
Phytochemical screening of solvents in extracted samples were carried out according to the standard methods for alkaloids, terpenoids, phenols, sugar, saponins, flavonoids, quinines, protein and steroids as described by [6]. Alkaloids were detected using Mayer’s reagent. The test was positive indicating the presence of alkaloids. Terpenoids was detected using concentrated sulphuric acid. The test was positive indicating the presence of terpenoids. Phenols were tested using ferric chloride. Phenols were positive for methanol, diethyl ether and N-butanol extracts. Sugars were tested using Fehling’s. Sugars were tested negative indicating the absence of sugars. Saponins were tested using distilled water. Frothing was observed indicating the presence of saponins. Flavonoids were tested using few fragments of magnesium ribbon and few drops of concentrated hydrochloric acid. Flavonoids were tested negative indicating the absence of flavonoids. Quinines were tested using sodium hydroxide reagent. Red colour is indicative of the presence of quinines. Proteins were tested using mercuric chloride or concentrated nitric acid reagent. Yellow colouration was observed which is indicative of the presence of proteins. Steroids were detected by Liebermann-Burchard test. Red colour was observed which is indicative of the presence of steroids.

2.3 Quantitative assays
In vitro DPPH radical scavenging assay was measured by the spectrophotometric method according to [7]. To a solution of DPPH in the tubes, 0.05 ml of extracts were added at different concentrations (250-500µg/ml). Control was also maintained. After 30 mins, the absorbance was read at 517nm. Ascorbic acid was used as a standard. Total amount of DPPH was expressed as µg/ml.

Reducing power of the extract was estimated spectrophotometrically by [8]. About 1 ml of water, methanol and chloroform extracts were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at then rapidly cooled and mixed with 2.5 ml of 10% trichloroacetic acid. 2.5 ml of distilled water and 0.5 ml of ferric chloride was added to it. The absorbance was measured at 700nm. Ascorbic acid was used as a standard. The reducing power was expressed as µg/ml.

The method described by [8] was used to determine the total antioxidant capacity of the extract. The tubes containing 2 ml of water, methanol and chloroform extracts, 1.8 ml of phosphomolybdenum reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) were incubated at 50°C for 90 mins. After the mixture cooled to room temperature, the absorbance was measured at 695nm. Ascorbic acid was used as the standard. The total antioxidant activity was expressed as mg equivalents of ascorbic acid per gram.

Hydroxyl radical scavenging activity was measured according to [9]. To a solution of hydrogen peroxide, 0.5 ml of water, methanol, and chloroform extracts and 0.9 ml of ethanol were added and incubated at room temperature for 10-15 mins. The absorbance was measured at 230nm. Gallic acid was used as the standard. The hydrogen peroxide assay was expressed as µg/ml.

In the scavenging activity was measured according to [10]. 1 ml of reaction mixture was taken in test tubes. The reaction mixture was prepared by adding 1 ml of 50m phosphate buffer solution, 0.075 ml of EDTA, 20M L-Methionine and 0.04 ml of 10mM Hydroxymide hydrochloride. The test sample of water, methanol and chloroform extracts were added to all these followed by a brief pre-incubation at 37°C for 10 mins. 50µl of 50mM Riboflavin was later added. The tubes were mixed well and exposed for 5 mins under UV fluorescent light. After the exposure time, 1 ml of Greiss reagent was added and the absorbance of the colour formed was read at 543nm. Greiss reagent was freshly prepared by mixing 1% sulphanilamide in 5% phosphoric acid. Gallic acid was used as the standard. The SOD activity was expressed as µg/ml.

Total phenolics assay was quantified and was expressed as gallic acid equivalent according to [6]. About 1 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent were added to the tubes having water, methanol, chloroform, diethyl ether and N-butanol and was incubated at room temperature for 3 mins. 2 ml of 20% sodium carbonate was added to this and kept at boiling water bath for 1 min. The blue colour formed was read at 650 nm.

In vitro amylase inhibition was studied by [8]. 1 ml of water, methanol and chloroform extracts were made to react with 0.2 ml of α amylase and 1 ml of 16mM sodium acetate buffer. After 20 mins incubation 1 ml of 0.1 % starch was added. After incubation for 5 mins 500µl of dinitrosalicylic acid was added. They were kept in a boiling water bath for 10-15 mins. The absorbance was recorded at 540nm. The percentage inhibition of α amylase was calculated using the formula

\[
\text{Inhibition (\%)} = 100(\text{control} - \text{test})/\text{control}
\]

The inhibitory effect of α glucosidase was carried out according to [11]. 1 mg of α glucosidase was dissolved in 100mL of phosphate buffer. The reaction mixture consisting of water, methanol and chloroform extracts were mixed with 1 ml of 2% starch which acts as a substrate. To these, 1 ml of 0.2 M Tris buffer was added. This was later incubated at room temperature for about 30 mins. After incubation 0.2 ml of α glucosidase dissolved in phosphate buffer was added. This was added to initiate the reaction. The reaction was terminated by the addition of 6N hydrochloric acid. The α glucosidase activity was determined spectrophotometrically at 540 nm. The inhibition rate (%) was calculated by the formula

\[
\text{α glucosidase Inhibition (\%)} = 100(\text{control} - \text{test})/\text{control} \times 100
\]

2.4 FT-IR
The water and methanol extract of *Artemisia Pallens* was treated with FTIR spectroscopy using Thermo Scientific FTIR. The FTIR analysis was carried out using [12].

2.5 GC-MS
GC-MS analysis of the methanolic extract of *A. pallens* was carried out using Thermo Gas Chromatograph DSQII series fitted with a DB35ms fused and a standard non-polar column.
(30mX 0.25mm, film thickness 0.25µm) coupled with 403 series mass detector with an injection volume of 1 µl. Helium was used as a carrier gas with a constant flow mode of 1.0 ml/min. The injector temperature was set at 260°C. The oven temperature of 70°C was raised to 260°C at 6°C/min. The GC-MS was carried out using [113].

2.6. Antimicrobial assay
The agar well diffusion method was used for the antibacterial assay. This was performed according to [14]. Petri dishes were prepared by pouring 15 of Muller Hinton Agar medium and allowed to solidify. The agar medium was solidified and E.coli, MRSA, P.aeruginosa, K.pneumoniae and B.subtilis were swabbed onto the medium uniformly. The inoculum was allowed to dry for 5 mins. The wells were made with sterilized stainless steel cork borer. The wells were labelled and each well was loaded with 300 µL of water, methanol and chloroform extracts. The plates were incubated in an incubator at 37°C for about 24 hrs. Antibacterial activity was determined by measuring the zones of inhibition.

3. Results and discussion

### Table 1: Results of the phytochemical analysis

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Tests</th>
<th>Water</th>
<th>Methanol</th>
<th>Diethyl Ether</th>
<th>N-Butanol</th>
<th>Chloroform</th>
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<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phenol &amp; Tannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Sugar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Quinines</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Protein</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ indicates presence and – indicates absence

Phytochemical screening of different solvent fractions of *A. Pallens* showed the presence of alkaloids, steroids, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinone, saponins and phenols as summarised in (Table 1).

In water extract alkaloids, terpenoids, saponins, quinines and proteins were present. In methanol alkaloids, terpenoids, phenols, quinines and steroids were present. In chloroform alkaloids, terpenoids, quinines and steroids were present. In diethyl ether, alkaloids and phenols were present and in N-butanol, alkaloids, phenols and quinines were present. According to [15], the *Artemisia pallens* is an aromatic herb found abundantly in humid habitats in the plains all over India.

### 3.1 DPPH radical scavenging activity

*Artemisia Pallens* shows DPH of water extract to be 50 µg/ml, methanol extract to be 60 µg/ml, chloroform extract to be 30 µg/ml, diethyl ether to be 50 µg/ml and N-butanol to be 60 µg/ml.

![Fig 2: Total DPPH radical scavenging activity of Artemisia Pallens](image)

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. With respect to [16], the DPHP radical scavenging activity of water, methanol and ethyl acetate extracts of *A.absinthium* were 7.37 µg/ml, 6.77 µg/ml and 41 µg/ml. This suggests that *A.pallens* has very good DPPH radical scavenging activity when compared to [16].

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. With respect to [17], the DPHP assay of the ethanol extract of *A. nilagirica* was 70µg/ml and the aqueous extract was 65µg/ml. This indicates that the DPHP activity of [17] was slightly higher than the DPH assay of *A.pallens*. DPHP assay also plays a role in investigating the radical scavenging activity of several natural compounds.

In the present study the water extract of *A.pallens* was 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. In [18], the DPHP assay of the aqueous extract of *A.argyi* was found to be 105µg/ml, ethanol extract was 107 µg/ml and methanol extract was 63 µg/ml. The DPHP radical scavenging activity of the ethanol and methanol extracts of *A.argyi* was slightly higher than *A.pallens* indicating that the nature of the phytochemicals seems to be more complex.

In the present study the water extract of my plant sample was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. The [19] DPHP analysis of the ethanol extract of *A.pallens* was found to be 150 µg/ml. This suggests that the DPHP radical scavenging activity of the ethanol extract appears to be better than all the extracts of *A.pallens*. The DPHP radical scavenging activity depends on the dosage of the plant extract used during the analysis.

In the present study the water With respect to [20], the DPHP assay of the methanol extract of *A.indica* was found to be 204 µg/ml. The water extract of *A.pallens* was found to be 50 µg/ml, methanol extract was 60 µg/ml, chloroform extract was 30 µg/ml, diethyl ether was 50 µg/ml and N-butanol was 60 µg/ml. There seems to be very good DPHP radical scavenging assay of *A.indica*. The extract of the plant appears to be a mixture where all the compounds may not possess DPHP radical scavenging activities.
In the present study the water extract of *A. pallescens* was found to be 50 μg/ml, methanol extract was 60 μg/ml, chloroform extract was 50 μg/ml, diethyl ether was 50 μg/ml and N-butanol was 60 μg/ml. In [21], the DPPH analysis of the petroleum ether extract of *A. biennis* was found to be 1314 μg/ml, dichloromethane extract was found to be 452 μg/ml, ethyl acetate extract was found to be 74 μg/ml and ethanol extract was found to be 67 μg/ml [19]. Apart from the petroleum ether and dichloromethane extracts of *A. biennis*, the ethyl acetate and ethanol extracts were slightly higher when compared to all the extracts of *A. pallellas*. This shows that all the extracts of *A. biennis* has good DPPH radical scavenging activity when compared to the DPPH assay of *A. pallellas*. 

In the present study the water the water extract of *A. pallellas* was found to be 50 μg/ml, methanol extract was 60 μg/ml, chloroform extract was 50 μg/ml, diethyl ether was 50 μg/ml and N-butanol was 60 μg/ml. According to [22], the radical scavenging activity of the essential oil *Artemisia amygdalina* was measured by the DPPH assay in-vitro. The highest activity (80.7%) was found at a concentration of 100 μg/mL. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This method has been used extensively to predict the antioxidant activity because of the relatively short time for analysis.

### 3.2 Antioxidant assay

*Artemisia Pallens* shows the antioxidant activity of water extract to be 198 μg/ml, methanol extract to be 748 μg/ml and chloroform extract to be 5 μg/ml.

![Antioxidant assay of Artemisia Pallens](image)

**Fig 3:** Antioxidant assay of *Artemisia Pallens*

In the present study the water extract of *A. pallescens* was 198 μg/ml, methanol extract was 748 μg/ml and chloroform extract was 5 μg/ml. With respect to [20], the methanol extract of *A. indica* was found to be equivalent to 298.80 μg of ascorbic acid. This indicates that *A. pallescens* has very good antioxidant assay. The antioxidant assay of the methanol extract of *A. pallescens* seems to be very good. This activity may be due to the presence of phytochemicals. Higher antioxidant activity is related to the ability to reduce DPPH. 

In the present study the water extract of *A. pallescens* was 198 μg/ml, methanol extract was 748 μg/ml and chloroform extract was 5 μg/ml. The [17], states that the total antioxidant activity of the ethanol and aqueous extract of *A. nilagirica* was 61.3 μg/ml and 64.7 μg/ml. These results indicate that antioxidants in *A. pallescens* seems to be excellent in water and methanol. These may be attributed to the presence of flavonoids and other specific phytochemicals corresponding to the antioxidant activity.

### 3.3 FRAP assay

FRAP assay of *Artemisia Pallens* shows the water extract to be 6 μg/ml, methanol extract to be 56 μg/ml and chloroform extract to be 12 μg/ml.

![FRAP assay of Artemisia Pallens](image)

**Fig 4:** FRAP reducing power assay of *Artemisia Pallens*

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6 μg/ml, 56 μg/ml and 12 μg/ml. In [20], the FRAP value of the methanol extract of *A. indica* was 13 μg/ml. These suggest that the FRAP values of *A. pallellas* seems to be excellent. These could be due to the presence of phenols and flavonoids. 

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6 μg/ml, 56 μg/ml and 12 μg/ml. With respect to [23], the FRAP assay of *A. selegeneis* of water extract was 46.2 μg/ml, ethanol extract was 29.4 μg/ml, N-butanol was 5.712 μg/ml, ethyl acetate was 7.1 μg/ml and petroleum ether was 0.612 μg/ml. These readings suggest that *A. pallellas* has very good reducing power. The FRAP can be applied to all the extracts of the different plants.

In the present study the FRAP assay of the water, methanol and chloroform extract of *A. Pallens* was 6 μg/ml, 56 μg/ml and 12 μg/ml. In [24], the FRAP assay of the ethanol extract of *A. annua* was 212 μg/ml. These results indicate that FRAP values of *A. annua* seems to be much better than the FRAP values of *A. pallellas*.

### 3.4 Hydrogen peroxide assay

Hydrogen peroxide assay of *Artemisia Pallens* of water extract was found to be 79 μg/ml, methanol extract was 13 μg/ml and chloroform extract was 13 μg/ml.

![Hydrogen peroxide assay of Artemisia Pallens](image)

**Fig 5:** Hydrogen peroxide assay of *Artemisia Pallens*

In the present study the hydrogen peroxide assay of water, methanol and chloroform of my sample extract was 79 μg/ml, 13 μg/ml and 13 μg/ml. From [19], the hydrogen peroxide assay of the ethanol extract of *A. pallellas* was 92.0 μg/ml. These results reveals that *A. pallellas* may have a potent hydrogen peroxide radical scavenging activity. These plants
may be used as an antioxidant in preventing the damage arising from hydroxyl radicals. In the present study the hydrogen peroxide assay of A. pallens was 79 µg/ml, 13 µg/ml and 13 µg/ml. With respect to [25], the hydrogen peroxide assay of the methanol extract of A. absinthium was 109 µg/ml. The results of [25] reveals that hydrogen peroxide assay was higher than that of A. pallens. The hydroxyl radicals of [25] has the capacity to modify every molecule of the living cell. The radicals may also damage the DNA leading to cancer.

3.5 SOD assay

SOD assay of Artemisia pallens of water extract was found to be 82%, methanol extract was 19% and chloroform extract was 94%.

In the present study the SOD analysis of the water, methanol and chloroform extract of A. pallens was 82%, 19% and 94%. The [25] states that the SOD assay of the methanol extract of A. absinthium was found to be 78%. The above said results shows that A. pallens has excellent SOD assay.

3.6 Phenols assay

Artemisia Pallens showed that the phenols present in water extract was found to be 117 GAE, methanol extract was 170 GAE, chloroform extract was 22 GAE, diethyl ether was 69 GAE and N-butanol was 14 GAE.

In the present study the 0.5 ml water extract of Artemisia Pallens was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186%.

3.7 Alpha amylase assay

Alpha amylase assay of 0.5 ml water extract of Artemisia Pallens was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186%.

In the present study the water, methanol, chloroform, diethyl ether and N-butanol extracts of A. pallens was 117 GAE, 170 GAE, 22 GAE, 69 GAE and 14 GAE. With respect to [27], the total phenol content of ethanolic extract of A. parviflora was found out to be 1.09 GAE. These suggest that the phenols present in all the extracts of A. pallens seems to be excellent. These components have good antioxidant activities of all plant materials. They are said to inhibit the effects of carcinogen in humans.

In the present study the water, methanol, chloroform, diethyl ether and N-butanol extracts of my sample extract was 117 GAE, 170 GAE, 22 GAE, 69 GAE and 14 GAE. In [28], the ethanol extract of A. pallens was 87.2 GAE. The water and methanol extract of A. pallens has a very high phenol content compared to the ethanol extract of A. pallens. These results indicate that this plant may be used to prepare a drug to prevent diabetes.

In the present study the 0.5 ml water extract of Artemisia Pallens was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml chloroform extract was 181% and 1 ml was 186%.

In the present study the 0.5 ml water extract of Artemisia Pallens was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186% respectively. According to [29], the ethanol extract of Artemisia herba-alba (Asso.) herbs and mucilage of 70% ethyl alcohol extract of Artemisia herba-alba (Asso.) showed the α- amylase assay to be 11% and 2%. The. These indicate that all the extracts of A. pallens was found to be higher than [29]. These enzymes seems to play a vital role in carbohydrate digestion.

In the present study the 0.5 ml water extract of Artemisia Pallens was found to be 121% and 1 ml was 11.5%, 0.5 ml methanol extract was 91% and 1 ml was 78%, and 0.5 ml of chloroform extract was 181% and 1 ml was 186% respectively. Following [30], the n-butanol and ethyl acetate extract of A. capillaris was 68% and 75%. These indicate that all the extracts of A. pallens was found to be higher than [30]. These findings suggest that the extracts from A. pallens can be used to treat postprandial hyperglycaemia.

3.8 Alpha glucosidase assay

Alpha glucosidase assay of 0.5 ml of water extract of Artemisia Pallens was found to be 55.8%, 1 ml was 58.8%, 0.5 ml of methanol extract was 30.8%, 1 ml was 51.8%, and 0.5 ml of chloroform extract was 27.8% and 1 ml was 56.8%.
In the present study the α-glucosidase assay of *A. pallens* of 0.5 ml of water extract was 55.8%, 1 ml was 58.8%, 0.5 ml of methanol extract was 30.8%, 1 ml was 51.8%, and 0.5 ml of chloroform extract was 27.8% and 1 ml was 56.8%. With reference to [30], the α-glucosidase assay of N-butanol extract of *A. capillaris* was 71%. These values indicate that all the values of *A. pallens* seems to be slightly lower than [30]. These values indicate that the extracts of *A. capillaris* could be used to cause a rise in post prandial blood glucose absorption.

3.9 FTIR

The FTIR analysis of methanol extract showed the presence of Furfuryl alcohol, benzy alcohol, Limonene, Ethyl benzy alcohol, phenyl phosphonic acid, beta butyrolactone, alpha bromo-p-xylene, triethyl phosphate and diethyl malonate which showed major peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45 and 3424.56.
In the present study of the FTIR analysis of the methanol and water analysis of *A. pallens* was found to contain alcohols, H-bonded alcohol, organic acid, ester, ether, Hydroxycarbocyclic acid, Oxime, ketone, alkaloid and natural ketone. This showed the peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45, 3424.56 725.40, 1109.73, 1247.05, 1370.56, 1588.61, 1612.04, 1651.11, 2134.99 and 3355.63 respectively. Around three peaks of *A. pallens* seems to be higher than the peaks of *A. annua* and many natural compounds seems to be present in *A. pallens*. These bioactive compounds are supposed to have good antioxidant properties. According to [31], the FTIR assay of the methanol extract of *Artemisia annua* showed the presence of alkenes, aliphatic fluoro components, alcohols, ethers, carboxylic acids, esters, nitro components, alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols showed major peaks at 777.31, 1028.06, 1155.36, 1315.45, 1417.68, 2848.86, 2918.30, 3273.20 and 3361.93 respectively.

### 4. GCMS

In the present study of the GCMS analysis of the methanol and water analysis of *A. pallens* water was found to contain alcohols, H-bonded alcohol, organic acid, ester, ether, Hydroxycarbocyclic acid, Oxime, ketone, alkaloid and natural ketone. This showed the peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45, 3424.56 725.40, 1109.73, 1247.05, 1370.56, 1588.61, 1612.04, 1651.11, 2134.99 and 3355.63 respectively. Around three peaks of *A. pallens* seems to be higher than the peaks of *A. annua* and many natural compounds seems to be present in *A. pallens*. These bioactive compounds are supposed to have good antioxidant properties. According to [31], the FTIR assay of the methanol extract of *Artemisia annua* showed the presence of alkenes, aliphatic fluoro components, alcohols, ethers, carboxylic acids, esters, nitro components, alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols showed major peaks at 777.31, 1028.06, 1155.36, 1315.45, 1417.68, 2848.86, 2918.30, 3273.20 and 3361.93 respectively.

### Table 3: FTIR activity of water extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Activity</th>
<th>Peak value</th>
<th>Intensity</th>
<th>Nature of compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACETALDOXIME</td>
<td>C2H5NO</td>
<td>59</td>
<td>Production of pesticides</td>
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<td>NONANONE</td>
<td>C9H18O</td>
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<td>Flavouring agent</td>
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<td>0.0473</td>
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<td>Flavouring agent</td>
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<td>1370.56</td>
<td>0.0519</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>5</td>
<td>2-Octanone</td>
<td>C18H6O</td>
<td>128</td>
<td>Flavour &amp; fragrance ingredient</td>
<td>1588.61</td>
<td>0.0981</td>
<td>Natural ketone</td>
</tr>
<tr>
<td>6</td>
<td>3-Octanone</td>
<td>C18H6O</td>
<td>128</td>
<td>Flavour &amp; fragrance ingredient</td>
<td>1612.04</td>
<td>0.132</td>
<td>Natural ketone</td>
</tr>
<tr>
<td>7</td>
<td>TRANS-2-HEXENE</td>
<td>C6H12</td>
<td>84</td>
<td>Biochemical for proteomic research</td>
<td>1651.11</td>
<td>0.251</td>
<td>Alkene</td>
</tr>
<tr>
<td>8</td>
<td>2-Nonanone</td>
<td>C9H18O</td>
<td>142</td>
<td>Food additives</td>
<td>2134.99</td>
<td>0.0720</td>
<td>Ketone</td>
</tr>
<tr>
<td>9</td>
<td>3-Nonanone</td>
<td>C9H18O</td>
<td>142</td>
<td>Flavouring agent</td>
<td>3355.63</td>
<td>1.032</td>
<td>Ketone</td>
</tr>
<tr>
<td>10</td>
<td>ACETALDOXIME</td>
<td>C2H5NO</td>
<td>59</td>
<td>Production of pesticides</td>
<td>725.40</td>
<td>0.246</td>
<td>Oxime</td>
</tr>
</tbody>
</table>

In the present study of the FTIR analysis of the methanol and water analysis of *A. pallens* was found to contain alcohols, H-bonded alcohol, organic acid, ester, ether, Hydroxycarbocyclic acid, Oxime, ketone, alkaloid and natural ketone. This showed the peaks at 722.90, 780.77, 1111.40, 1247.14, 1301.67, 1422.37, 1485.18, 1551.65, 1566.45, 3424.56 725.40, 1109.73, 1247.05, 1370.56, 1588.61, 1612.04, 1651.11, 2134.99 and 3355.63 respectively. Around three peaks of *A. pallens* seems to be higher than the peaks of *A. annua* and many natural compounds seems to be present in *A. pallens*. These bioactive compounds are supposed to have good antioxidant properties. According to [31], the FTIR assay of the methanol extract of *Artemisia annua* showed the presence of alkenes, aliphatic fluoro components, alcohols, ethers, carboxylic acids, esters, nitro components, alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols showed major peaks at 777.31, 1028.06, 1155.36, 1315.45, 1417.68, 2848.86, 2918.30, 3273.20 and 3361.93 respectively.
# Table 4: GCMS analysis of *Artemisia pallens*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Compound nature</th>
<th>Pharmacological action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hotrienol</td>
<td>C10H16O</td>
<td>152</td>
<td>Alkaloid</td>
<td>Flavour and fragrance agent</td>
</tr>
<tr>
<td>2</td>
<td>18,19-Secoyohimban-19-oic acid,</td>
<td>C21H24N2O3</td>
<td>352</td>
<td>Essential oil</td>
<td>Anti-Microbial and antiviral</td>
</tr>
<tr>
<td>3</td>
<td>Lilac alcohol</td>
<td>C10H18O2</td>
<td>170</td>
<td>Alkaloid</td>
<td>Flavour and fragrance agent</td>
</tr>
<tr>
<td>4</td>
<td>Paromomycin</td>
<td>C23H45N5O1</td>
<td>615</td>
<td>Antibiotic</td>
<td>Including amoebiasis, giardiasis, Leishmaniasis, and tapeworm infection</td>
</tr>
<tr>
<td>5</td>
<td>Davanone</td>
<td>C15H24O2</td>
<td>236</td>
<td>Essential oil</td>
<td>Perfumes, flavours and cosmetics, anti-insects</td>
</tr>
<tr>
<td>6</td>
<td>1-Eicosanol (CAS)</td>
<td>C20H42O</td>
<td>295</td>
<td>Straight chain fatty acid</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>7</td>
<td>2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-</td>
<td>C11H12O4</td>
<td>208</td>
<td>Unsaturated carboxylic acid</td>
<td>Anti tumor and anti-microbial</td>
</tr>
<tr>
<td>8</td>
<td>n-Hexadecanoic acid</td>
<td>C16H32O2</td>
<td>256</td>
<td>Palmitic acid</td>
<td>Antioxidant 5 Alpha reductase inhibitor Pesticide Nematicide Lubricant Hypcholesterolemic Antiandroaginic</td>
</tr>
<tr>
<td>9</td>
<td>Ascorbic acid</td>
<td>C38H68O8</td>
<td>652</td>
<td>Essential acid</td>
<td>Vitamin C</td>
</tr>
<tr>
<td>10</td>
<td>Formic acid</td>
<td>C15H24O3</td>
<td>252</td>
<td>Essential acid</td>
<td>A major use of formic acid is as a preservative and antibacterial agent in livestock feed. Formic acid arrests certain decay processes and causes the feed to retain its nutritive value longer, and so it is widely used to preserve winter feed for cattle.</td>
</tr>
<tr>
<td>11</td>
<td>Isophytol</td>
<td>C20H40O</td>
<td>296</td>
<td>Essential oil</td>
<td>Perfumes, flavours and cosmetics, anti-insect and anti-bacterial</td>
</tr>
<tr>
<td>12</td>
<td>Gamolenic Acid</td>
<td>C18H30O2</td>
<td>278</td>
<td>Essential oil</td>
<td>Used for rheumatoid arthritis (RA), polyps in the mouth, high cholesterol and other blood fats, heart disease, metabolic syndrome (Syndrome-X), diabetic nerve pain, attention deficithyperactivity disorder (ADHD), depression, depression after childbirth, chronic fatigue syndrome (CFS), and hay fever (allergic rhinitis). Some people use it to prevent cancer and to help breast cancer patients respond faster to treatment with the drug tamoxifen.</td>
</tr>
</tbody>
</table>

---

**Graph**

1. **Hotrienol**
   - NL: 9.99E2 GC-403200 7.07 1 6.00 5 6.75E4 + e Full ms [50.00-650.00]
2. **18,19-Secoyohimban-19-oic acid,**
   - NL: 18,19-Secoyohimban-19-oic acid, 18,17,20,21-tetradecahydro-16-thioxyximinothiyl methyl ester (15a,16c)
3. **Lilac alcohol**
   - NL: 9.99E2 SI 661, 906, mannitol, Entry 18464, CAS 33081-37-7
   - NL: 9.99E2 SI 200, 256, mannitol, Entry 18464, CAS 33081-37-7 Lilac alcohol D
<table>
<thead>
<tr>
<th>Page</th>
<th>Compound</th>
<th>Data</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Paromomycin</td>
<td>GC-403486 12.93</td>
<td>1: 6.00, 5: 1.44E5 + c Full ms [50.00-650.00]</td>
</tr>
<tr>
<td>5</td>
<td>Davanone</td>
<td>GC-403634 15.97</td>
<td>1: 6.00, 5: 2.71E7 + c Full ms [50.00-650.00]</td>
</tr>
<tr>
<td>6</td>
<td>1-Eicosanol (CAS)</td>
<td>GC-403768 18.72</td>
<td>1: 6.00, 5: 9.5E5 + c Full ms [50.00-650.00]</td>
</tr>
<tr>
<td>7</td>
<td>2-Propenoic acid, 3-(2,3-dimethoxyphenyl) -</td>
<td>GC-403558 14.41</td>
<td>1: 6.00, 5: 7.04E6 + c Full ms [50.00-650.00]</td>
</tr>
<tr>
<td>8</td>
<td>n-Hexadecanoic acid</td>
<td>GC-403963 22.70</td>
<td>1: 6.00, 5: 1.86E6 + c Full ms [50.00-650.00]</td>
</tr>
</tbody>
</table>
In the present study of the methanol extract of *Artemisia pallens* showed the presence of many essential compounds like ascorbic acid, formic acid, lilac alcohol, Paromomycin, Hotrienol, Isophytol, 18,19-Secoyohimban-19-oic acid, Davanone, 1-eicasonol, 2-Propenoic acid, 3-(2,3-dimethoxyphenyl), n-Hexadecanoic acid and Gamolenic acid. Some of these compounds are used in pharmaceuticals. According to [22], the essential oil of *A.amygdalina*, showed the presence of Sabinene (14.3%), p-cymene (12.5%), Eucalyptol (16.7%), and L-borneol (12%).

**4.1 Antibacterial activity**

![Photo of Microorganism used](image-url)
Table 5: Microorganisms used to study the antimicrobial activity of *A. pallens*

<table>
<thead>
<tr>
<th>Organism name</th>
<th>Disc</th>
<th>Water</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>4 mm</td>
<td>14 mm</td>
<td>15 mm</td>
<td>18 mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>10 mm</td>
<td>8 mm</td>
<td>9 mm</td>
<td>2 mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>K.pneumoniae</em></td>
<td>12 mm</td>
<td>11 mm</td>
<td>12 mm</td>
<td>3 mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8 mm</td>
<td>10 mm</td>
<td>5 mm</td>
<td>8 mm</td>
<td>Nil</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Nil</td>
<td>5 mm</td>
<td>5 mm</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

According to the present study of *Artemisia Pallens*, the water, methanol and chloroform extract was found to be very effective against various bacteria as indicated by the zone of inhibition. Maximum inhibition was obtained by the chloroform extract of *Artemisia Pallens* against *Bacillus subtilis* (18mm), followed by methanol extract and the water extract, methanol extract of *K. pneumoniae* (12mm) and water extract of *K. pneumoniae* (11mm).

According to [18], the ethanolic extract of *A. pallens* revealed the tested organisms like *Bacillus lentus* and *salmonella paratyphi* showed sensitivity response towards plant extract containing saponin subsequently. Other organism such as *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* were considered as moderate sensitivity in response to the saponin content in the plant sample extract when compared with ciprofloxacin. The results showed the zone of inhibition of the100 μg of sample extract when compared with 10 μg of standard were susceptible to antibiotic which shows the sample sensitivity against antibiotic (ciprofloxacin disc).

5. Conclusion

*Artemisia Pallens* showed the presence of alkaloids, steroids, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinone, and phenols. The *Artemisia Pallens* leaves showed significant antioxidant activity against the standard. It also revealed the significant DPPH radical scavenging assay, total phenols assay, FRAP reducing power, Hydroxyl radical scavenging activity and also showed significant Super oxide radical scavenging activity, alpha amylase, alpha glucosidase, GCMS, FTIR and antibacterial assays.

6. Acknowledgement

The author is thankful to the director and also to the staff of CBNR for providing the necessary support to carry out this work.

7. References

20. Mahpara Qadir, Javid A. Banday, GC-MS analysis and Antioxidant activity of essential oil of Artemisia


23. Feng Shi, Xiaobin Jia, Chenglei Zhao and Yan Chen, Key Laboratory of Delivery Systems of Chinese Medicine, Jiangsu Provincial Academy of Chinese Medicine, Jiangsu, Nanjing, China, Antioxidant Activities of Various Extracts from Artemisia selengensis Turecz (LuHao), Molecules. 2010; 15:4934-4946.


