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Evaluation of pharmacological activity of Artemisia pallensby Antibacterial and Antioxidant activity: An invitro study

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Abstract

Plants are a rich source of therapeutic compounds that have tremendous applications in the pharmaceutical industry. Artemisia pallens is a plant that has long been utilized in traditional medicine as a treatment for certain diseases. This study aims to analyse the phytochemicals as plant secondary metabolites, evaluate the antioxidant and antibacterial activities of A. pallensespecially in the four different districts of Karnataka region.Phytochemical analysis was performed to investigate the plant's secondary metabolites such as Saponins, Flavonoids, Cardiac Glycosides, Terpenoids, Alkaloids, Steroids and Carbohydrates. The methanol extracts of the plants were used to evaluate the in vitro antioxidant activity by using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical and the antibacterial activity against gram positive and gram-negative bacteria. Leaf extract A. pallens was observed to have the highest antioxidant activity with IC50 value as compared to the standard Butylated Hydroxy Toluene (BHT). The leaf extracts of four different regions also showed good potential zone of inhibition against Escherichia coli and Pseudomonas syringae which are Gram negative in nature. Only two samples showed good zone of inhibition against Bacillus subtilis and Staphylococcus aureus which are Gram positive in nature. Methanol extract ofA. pallens showed the best 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity. Two samples showed that the leaf extracts have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants pallens extracts contain phytochemicals which are capable of mitigating against chronic health conditions such as cancer.

Keywords: Artemisia pallens, Phytochemistry, Antibacterial activity, Antioxidant activity, Secondary metabolites, Cancer.

Introduction

In addition to providing humans with vital nutrients, plants also contain biologically active compounds that are good for human health and the treatment of a variety of diseases[1]. They contain a variety of compounds which includes lipids, phytochemicals, pharmaceuticals, flavours, and fragrances and have been used in the food, pharmaceutical, and cosmetics industries.Phytochemical substances are the fundamental rationale for using plants in traditional medical practices, and in developing nations like India, traditional medicine is the main source of the healthcare system[2]. Traditional medicine is widely used in India, particularly in some of the regions. This may be because the area has a vast range of flora and also to the people living there have a relatively low socioeconomic status[3]. Many bioactive compounds found in medicinal plants have the ability to be antioxidants, antimicrobials, anticancer agents, and anti-inflammatory agents. The majority of plant-derived medications are made from crude extracts, which are used to treat both viral and chronic illnesses and contain a complex mixture of different phytochemicals[4]. A large number of secondary metabolites are found in many different plant species, but only a small number of these have been investigated and shown to represent a significant source of bioactive compounds. Creating appropriate screening processes is essential for both quality control and the search for new compounds[5]. Certain drugs with a high activity profile have been delivered as a result of the extraction and characterisation of these bioactive compounds[6].

Artemisia pallens, Davana is an aromatic herb from the Asteraceae family that is endemic to India and other tropical regions. It is a high-value annual fragrant herb cultivated commercially in south India as a short-season bloom from November to March. India had a monopoly on the production and export of davana oil. Mainly it is often employed in religious rituals and in the creation of garlands, bouquets, flower arrangements, and floral chaplets, gives religious events a touch of freshness and a rich, delicious scent.

In Ayurveda, *A pallens* is used to treat measles, colds, coughs, depression, diabetes, and high blood pressure. The powdered leaf has long been used as an antidepressant, antihypertensive, and antidiabetic medication. The presence of naturally occurring bioactive compounds accounts for the therapeutic potential. The present study mainly focusing on screening of antibacterial activity and antioxidant activity of leaf extract especially in four different districts of Karnataka state.

Materials and Methods

Plant sample *Artemisia pallens*were collected from four different districts of Karnataka state (Chikkaballapur, Chitradurga, Bangalore rural and Shivamogga).

Surface sterilization of Plant material

After collecting the plant samples, the adhering material was removed by washing them under running tap water and then again with sterile distilled water. The leaves were subjected to a series of aseptic procedures, including immersion in 70% ethanol for one minute and mercuric chloride (1 mg/1 ml) for ten minutes, before being washed with sterile distilled water.

Preparation of Extracts

After washing the leaves samples were kept for air drying under shade at room temperature. The dried leaves were then crushed into fine powder (in blender) and stored in an airtight container until further use. The shade dried powder (10g) was extracted with 100 ml of methanol solvent using Soxhlet apparatus. The dried residue was stored in desiccators until further use.

Phytochemical analysis

The aqueous methanol leaf extract of *A. pallens* were used for phytochemical screening. Screening for the presence of different phytochemicals was carried out in accordance with the standard protocols to identify Saponins, Flavonoids, Cardiac Glycosides, Terpenoids, Alkaloids, Steroids and Carbohydrates[7,8].

Test for Saponins

5 ml of distilled water were added to 1ml of the leaf extract in a test tube, and the mixture with continuous stirring. The presence of saponins is confirmed by the formation of froth that persists for 30 minutes.

Test for Flavonoids

To 1ml of the extract, 10 ml of ethyl acetate was added and then heated over a water bath for 3 minutes. The plant extract and ethyl acetate solution were cooled and filtered. After that, 4 ml of the filtrate and 1 ml of diluted ammonia solution were shaken together. The layers were allowed to separate, and the yellow colour in the ammonia layer indicated the presence of flavonoids.

Test for Cardiac Glycosides (Keller-Killiani Test)

2 ml of glacial acetic acid containing 1 drop of ferric chloride solution was added to 1ml of the extract diluted to 5 ml in water. 1ml of Sulfuric acid were added. A brown ring at the interface suggested that cardenolides, which have a deoxysugar property. In the acetic acid layer, a greenish ring may emerge slightly above the brown ring and progressively expand throughout this layer, while a violet ring may appear below the brown ring.

Test for Terpenoids

2 ml of chloroform was added to 1ml of the extract. Then, 3 mL of pure sulfuric acid was carefully added to form a layer. The presence of terpenoids in the plant extract was indicated by a reddish-brown colouring.

Test for Alkaloids

In a test tube 5 ml of 1% HCl and 1ml of extract were added and kept in water bath. Few drops of Mayer's reagent were added to one millilitre of the filtrate, and Dragendorff's reagent was added to another millilitre in a similar manner. Turbidity or precipitation with both reagents was used as preliminary evidence for the presence of alkaloids.

Tests for steroids:

Liebermann-Burchard's test: 1ml of extract was mixed with 1 ml of chloroform. To the mixture, 1 ml acetic anhydride was added followed by addition of 2 ml of conc. sulphuric acid from the sides of test tube and observed for the formation of red-violetcolored layer at junction for presence of steroids.

Test for tannins

a) **Ferric chloride test:** Few drops of 5% $FeCl_3$ was added to 2-3 ml of extract and observed for deep blue-black color.
b) **Gelatin test:** Gelatin solution (1%) containing 10% sodium chloride was added to 1 ml of extract and observed for formation of precipitate.

Test for Carbohydrates:
Benedict's test: To the extracts, equal volume of Benedict's reagent was added and heated in boiling water bath for 5 min. The appearance of green, yellow, or red color indicates presence of reducing sugar.

Antibacterial activity
Antibacterial activity is one of the qualitative technique which is used for screening the bioactive compounds which are present in the medicinal plants. Leaf extracts from four different districts are used for the antibacterial activity against a wide spectrum of Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus* and *Knoellia sinensis*) and Gram negative bacteria (*Escherichia coli, Pseudomonas syringae* and *Pseudomonas aeruginosa*).

Disc diffusion assay:
Disc diffusion method is used to test the antibacterial activity of all the four samples from four different districts. The samples were inoculated into MHA (Muller Hinton Agar) plates by using sterile cotton swabs. Approximately 5mm in diameter, wells were prepared by using cork borer and around 200µl of methanol extract samples were added into it. Then, the plates were placed in bacterial incubator for 48hrs around 37°C for the development of zone of inhibition[9]. Streptomycin is used as a positive control for all the four samples. Zone of inhibition was calculated after the incubation period[10, 11]. The experiment is repeated for all the four samples in triplicates.

Antioxidant activity
DPPH Assay
1,1-diphenyl-2-picryl-hydrazyl (DPPH) was used to test the extract's capacity to scavenge free radicals[12]. Extract concentration around 0.1 mg/ml in 4 mL of methanol was mixed with 1 mL of methanol solution containing DPPH radicals of 0.2 mM. The mixture was shaken vigorously and allowed to stand for 30 min in the dark chamber. At 517 nm, the absorbance was measured against a blank. Through interpolation from linear regression analysis, the IC50 value was determined. Butylated hydroxy toluene (BHT) was used as a standard. The capacity of radical scavenging activity was calculated using the following equation:
DPPH scavenging effect (%) = [{A0 – A1} A0] × 100
Where, A0 is the absorbance of the control reaction and A1 is the absorbance of the presence of the sample.

Result
Collection of Plant Material
Plant material, *A. pallens* was collected from four different districts of Karnataka, India. And identified by the scientist Prof. Y L Ramachandra, Senior Professor, Department of Biotechnology and Bioinformatics, Kuvempu University, Shivamogga district, Karnataka (Fig 1-4).

Fig 1: Medicinal plant *A. pallens* used in the present study collected from Bangalore Rural region.

Fig 2: Medicinal plant *A. pallens* used in the present study collected from Chikkaballapur.

Fig 3: Medicinal plant *A. pallens* used in the present study collected from Chitradurga.

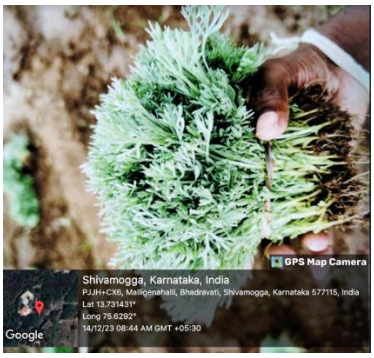


Fig 4: Medicinal plant *A. pallens* used in the present study collected from Shivamogga.

Preparation of Extracts

The leaves were dried and crushed into a fine powder for the further extraction process using Soxhlet apparatus and further used for analytical techniques (Fig 5-7).

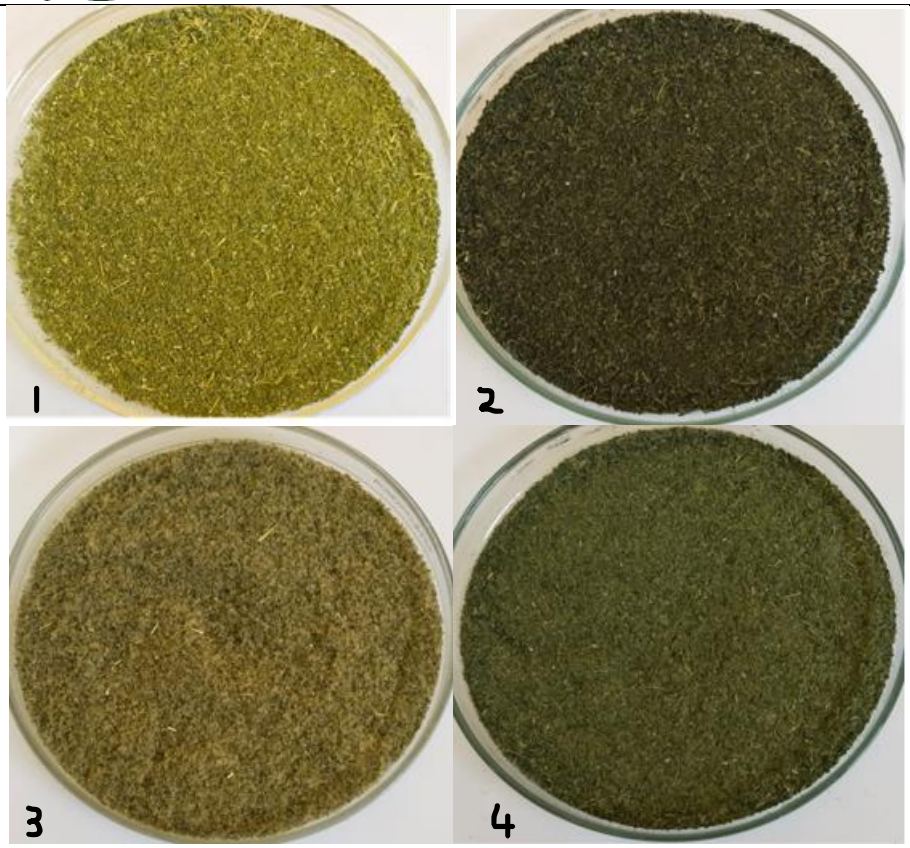


Fig 5: Coarse powder of selected plant (four districts)- Leaves

Bangalore rural 2. Chikkaballapur district 3. Chitradurga district 4. Shivamogga district.

Fig 6 Soxhlet extraction apparatus used for extraction process.

Fig 7: Methanol extracts of leaf from four different districts which are used for different analytical techniques.

Phytochemical analysis

Qualitative phytochemical analysis of leaf extract of *A. pallens* exhibited distinguishable results by showing the presence of many significant phytoconstituents. Saponins, Flavonoids, Terpenoids, Alkaloids were the prominent phytochemicals were present in all the four samples. Although all the four samples were showed more or less efficient (as observed from the intensity of color) for the production of secondary metabolites (Table 1 & Fig 8(a-g)).

Table 1: Phytochemical analysis of methanol extract of the medicinal plant *A. pallens* of 4 different districts

Parameter	Sample 1 Bangalore Region	Sample 2 Chikkaballapur District	Sample 3 Chitradurga District	Sample 4 Shivamogga District
Test for Saponins	+	+	+	+
Test for Flavonoids	+	+	+	+
Test for Cardiac Glycosides	-	-	-	-
Test for Terpenoids	+	+	+	+
Test for Alkaloids	+	+	+	+
Test for Steroids	+	-	-	+
Test for Carbohydrates	-	-	+	-

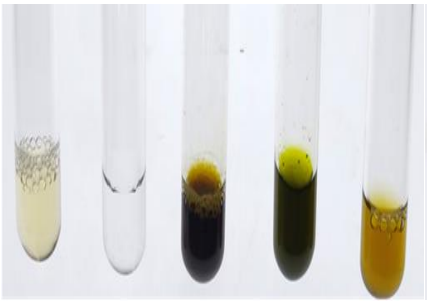


Fig 8(a): Test for Saponins

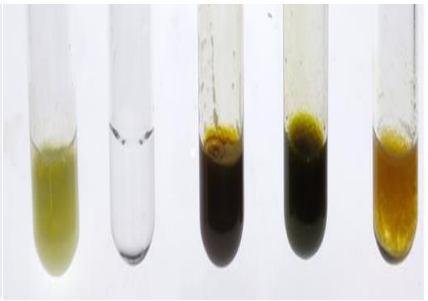


Fig 8(b): Test for flavonoids

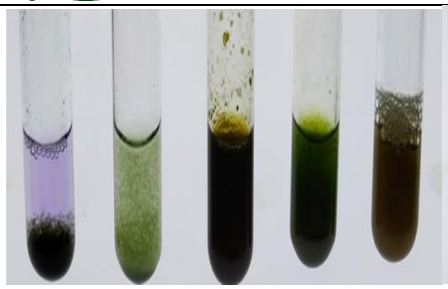


Fig: 8(c): Test for Cardiac Glycosides

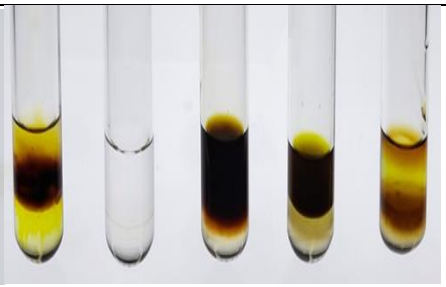


Fig 8(d): Test for Terpenoids

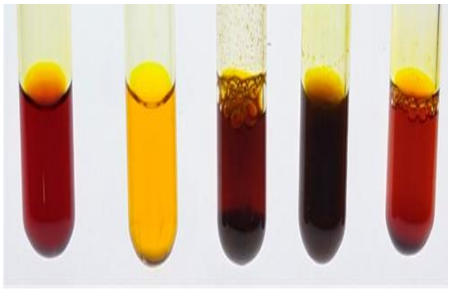


Fig 8(e): Test for Alkaloids

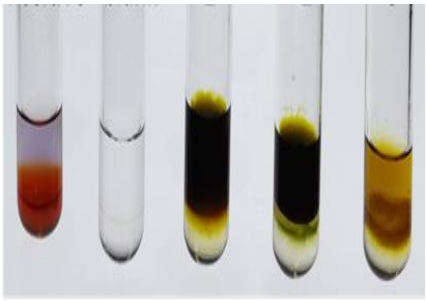


Fig 8(f): Test for Steroids

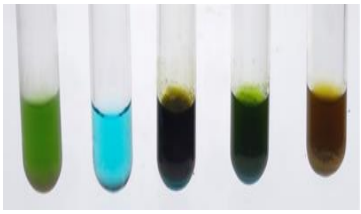
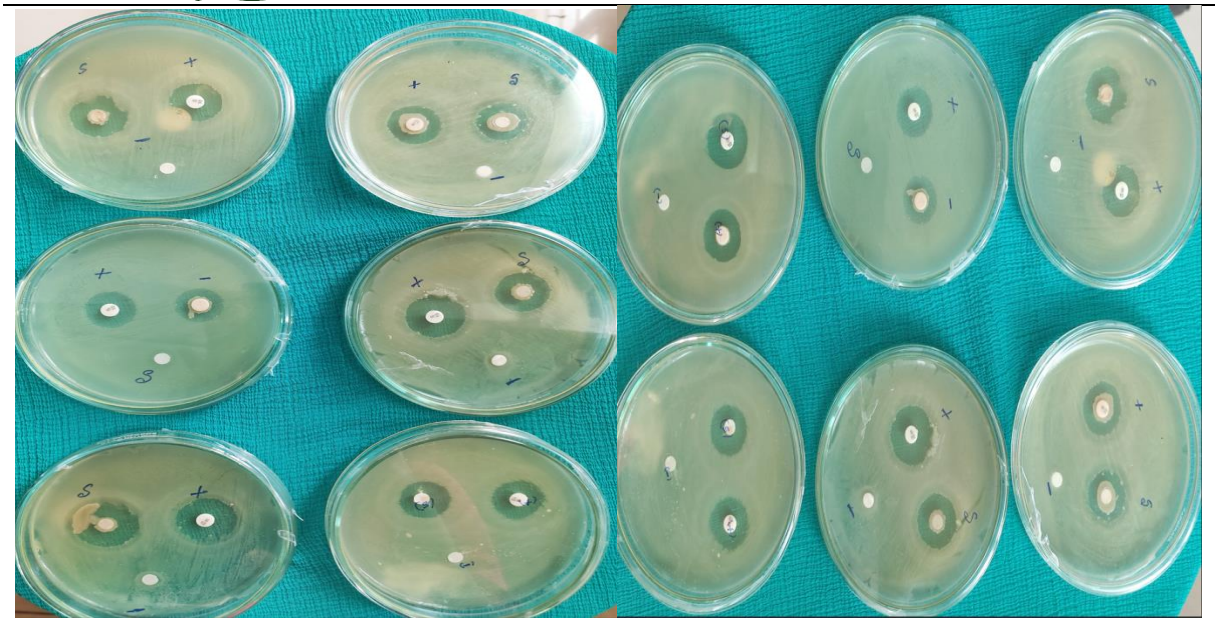


Fig 8(g): Test for Carbohydrates

Antibacterial activity:

Leaf extracts of *A. pallens* were screened for antibacterial activity. All the four samples showed good potential zone of inhibition against *Escherichia coli* and *Pseudomonas syringae* which are Gram negative in nature. Only two samples (S2 & S3) showed good zone of inhibition against *Bacillus subtilis* and *Staphylococcus aureus* which are Gram positive in nature (Figure 9 and Table 2).

Sample 1: Bangalore rural Sample 2: Chikkaballapur District



Sample 3: Chitradurga District Sample 4: Shivamogga District

Fig 9: Antibacterial activity of four different samples determined by disc diffusion method against standard antibiotic Streptomycin.

Table 2: Antimicrobial activity showing zone of inhibition.

Test Sample	*E. coli* (mm)	*P. aeruginosa* (mm)	*P. syringae* (mm)	*B. subtilis* (mm)	*S. aureus* (mm)	*K. sine* (mm)
Sample 1	15	10	20	15	10	13
Standard	23	22	20	21	23	20
Sample 2	14	9	18	18	19	11
Standard	22	21	20	20	21	21
Sample 3	16	10	20	17	18	12
Standard	22	20	20	21	20	20
Sample 4	15	8	17	13	15	10
Standard	22	21	20	19	20	19

Antioxidant activity

DPPH assay

The antioxidant activity of leaf extract of all the four samples were measured by the ability of scavenging DPPH free radicals, was compared with standardButylated Hydroxy Toluene (BHT). As antioxidants donate proton to DPPH radicals, theabsorption decreases. The reduction in the number of DPPH molecules can be correlatedwith the number of available hydroxyl groups. The antioxidant activity of four leaf extracts was determined and the results are tabulated in (Figure 10-13 and Table 3-6). Sample 2 & 3 showed that the leaf extracts have the proton donating ability and could serve as free radicalinhibitors or scavenging, acting possibly as primary antioxidants (since R^2 value is close to 1) (Fig. 8.5).

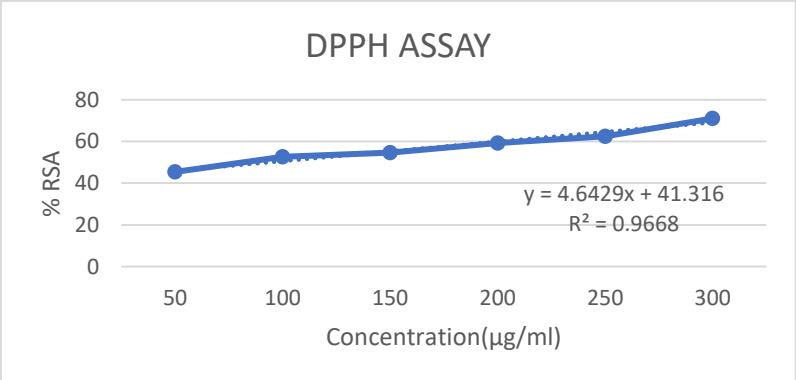


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Fig 10: Sample 1: Bangalore region -DPPH Scavenging activity of leaf extract

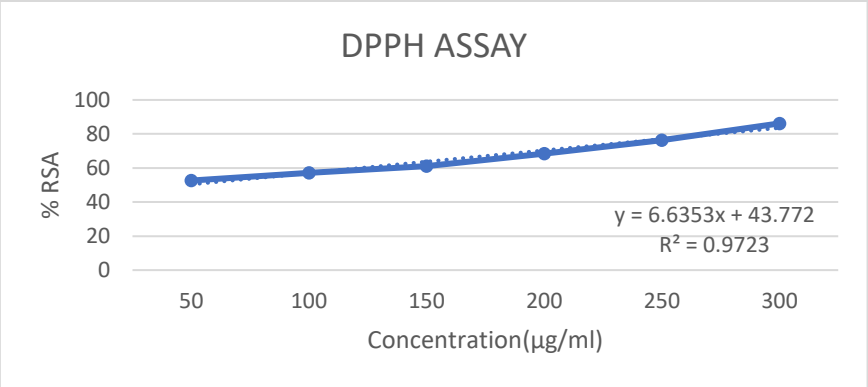

Fig 11: Sample 2: Chikkaballapur District-DPPH Scavenging activity of leaf extract

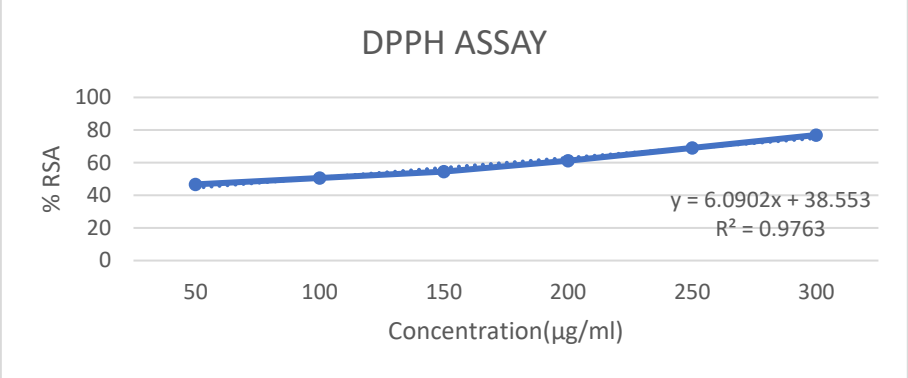

Fig 12: Sample 3: Chitradurga District-DPPH Scavenging activity of leaf extract

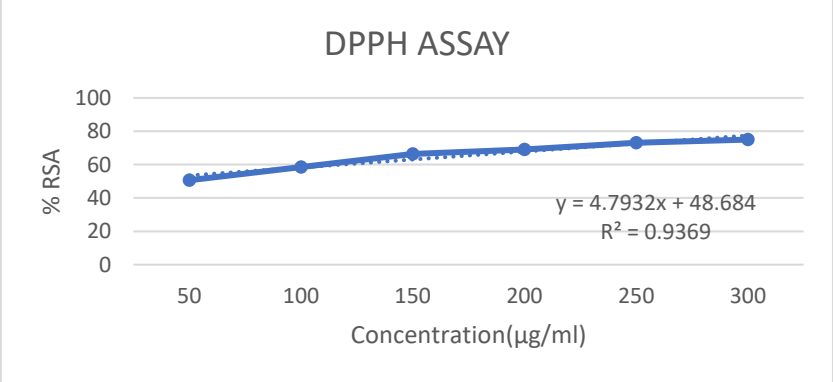

Fig 13: Sample 4: Shivamogga District-DPPH Scavenging activity of leaf extract

Table 3: Sample 1: Bangalore region -DPPH radical scavenging values of leaf extract

Calculation of % Radical Scavenging and IC50 from DPPH Assay				
Absorbance Measurement Data				
Concentration(µg/ml)	**control**	**sample 1**	**%RSA**	**IC50**
50	1.52	0.83	45.39473684	1.870382735
100	1.52	0.72	52.63157895	12.6395141
150	1.52	0.69	54.60526316	23.40864546
200	1.52	0.62	59.21052632	34.17777682
250	1.52	0.57	62.5	44.94690818
300	1.52	0.44	71.05263158	55.71603954

Table 4:Sample 2: Chikkaballapur District-DPPH radical scavenging values of leaf extract

Calculation of % Radical Scavenging and IC50 from DPPH Assay				
Absorbance Measurement Data				
Concentration(µg/ml)	**control**	**sample 4**	**%RSA**	**IC50**
50	1.52	0.72	52.63157895	0.938616189
100	1.52	0.65	57.23684211	8.474070502
150	1.52	0.59	61.18421053	16.00952481
200	1.52	0.48	68.42105263	23.54497913
250	1.52	0.36	76.31578947	31.08043344
300	1.52	0.21	86.18421053	38.61588775

Table 5: Sample 3: Chitradurga District-DPPH radical scavenging values of leaf extract

Calculation of % Radical Scavenging and IC50 from DPPH Assay				
Absorbance Measurement Data				
Concentration(µg/ml)	**control**	**sample 3**	**%RSA**	**IC50**
50	1.52	0.81	46.71052632	1.879577025
100	1.52	0.75	50.65789474	10.08948803
150	1.52	0.69	54.60526316	18.29939903
200	1.52	0.59	61.18421053	26.50931004
250	1.52	0.47	69.07894737	34.71922104
300	1.52	0.35	76.97368421	42.92913205

Table 6: Sample 4: Shivamogga District-DPPH radical scavenging values of leaf extract

Calculation of % Radical Scavenging and IC50 from DPPH Assay				
Absorbance Measurement Data				
Concentration(µg/ml)	**control**	**sample 2**	**%RSA**	**IC50**
50	1.52	0.75	50.65789474	0.27455562
100	1.52	0.63	58.55263158	10.70600017
150	1.52	0.51	66.44736842	21.13744471
200	1.52	0.47	69.07894737	31.56888926
250	1.52	0.41	73.02631579	42.00033381
300	1.52	0.38	75	52.43177835

Conclusion:

Based on the above results, the plant extracts showed a positive result towards antibacterial activity and antioxidant activity, henceforth they can be used in the treatment of infectious diseases. Phytochemical screening of crude methanolic extracts of *A. pallens* confirmed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids. Methanolic extract of the leaf also showed moderate bacterial growth inhibition as well as antioxidant activity. The observed antibacterial and antioxidant activities support the traditional use of this plant for the treatment of various ailments. Therefore, *A.pallens*leaf can be a potential source in search of potent natural antioxidants.

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