Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
Impact Factor (RJIF): 6.35
www.phytojournal.com
JPP 2025; 14(5): 08-15
Received: 04-06-2025
Accepted: 10-07-2025

Ashwini Mulay
Department of Botany, New Arts, Commerce &Science College, Ahilyanagar, Maharashtra, India

Yogesh Gahile
Department of Botany, New Arts, Commerce &Science College, Ahilyanagar, Maharashtra, India

Tukaram Nikam
Department of Botany, Savitribai Phule Pune University, Pune, Maharashtra, India

Digamber Ahire
Department of Botany, New Arts, Commerce &Science College, Ahilyanagar, Maharashtra, India

Corresponding Author:
Yogesh Gahile
Department of Botany, New Arts, Commerce &Science College, Ahilyanagar, Maharashtra, India

Phytochemical and pharmacological study of medicinally important plant *Artemisia pallens* wall

Ashwini Mulay, Yogesh Gahile, Tukaram Nikam and Digamber Ahire

DOI: https://doi.org/10.22271/phyto.2025.v14.i5a.15544 N

Abstract
Artemisia pallens is a medicinally important plant traditionally used for its antimicrobial, antidiabetic and and antioxidant properties. The present study was undertaken to evaluate the phytochemical profile and pharmacological potentials of whole plant biomass of *Artemisia pallens* with a focus on solvent-dependant variations.
Plant material was extracted using solvents of varying polarity (distilled water, ethanol, methanol, petroleum ether and chloroform). Quantitative estimation of phenols, flavonoid, tannins and artemisinin was carried out using standard colorimetric methods using spectrophotometer. Pharmacological evaluation included antioxidant evaluated through DPPH radical scavenging assay and hydroxyl radical scavenging assay, while antimicrobial assay was performed against different bacterial strains.
Phytochemical analysis revealed that the presence of phenols was significantly higher in distilled water extract and flavonoids and tannin content were significantly higher in methanolic extracts. Artemisinin content was significantly high in ethanolic extract (1.01 ± 0.04 mg/gm dry biomass). A strong positive correlation was observed between phenolic, flavonoid and tannin levels with both artemisinin content and antioxidant activity.
The study highlights *A. pallens* as a rich source of bioactive phytoconstituents with strong antioxidant potential. Solvent selection significantly influences phytochemical yield and pharmacological activity, suggesting that optimization of extraction methods can enhance therapeutic efficacy.

Keywords: *Artemisia Pallens*, Secondary metabolites, Antioxidant activity, antimicrobial activity, plant growth regulators (PGR's)

Introduction
Artemisia pallens Wall. (Ex DC.) is indigenous species of India belonging to family Asteraceae commonly known as davana. Davana is cultivated in Maharashtra and South Indian states like Karnataka, Andhra Pradesh, Tamil Nadu. It is widely used in traditional systems of medicine including Siddha, Ayurveda, and Unani for its diverse therapeutic properties. The plant is known for its antimicrobial, antioxidant, antidiabetic, and anti-inflammatory activities, which are attributed to its rich content of secondary metabolites.
Flavonoids, tannins, terpenoids, alkaloids, phenolic compounds, and essential oils are among the bioactive substances found in *Artemisia pallens*, according to phytochemical investigations(Kumar & Kumud, 2010) [9]. Among these, substances with significant pharmacological effects, including davanone, linalool, and caryophyllene, have been found in its volatile oil portion (Bail *et al*., 2008) [3].
The presence of tannins in different extracts has also been measured recently; ethyl acetate extracts had a higher tannin content (~102.76 mg/g) than n-hexane and chloroform extracts (Suresh, Singh, *et al*., 2011a) [15].
Also, previous pharmacological studies have confirmed the traditional use of A. pallens. using DPPH and FRAP assays, for example, research has validated its antioxidant activity (Suresh, Suresh, *et al*., 2011) [17] and *in vivo* models, ethanolic extracts have been shown to drastically lower blood glucose levels (Pavithra *et al*., 2020) [12].
A thorough investigation that uses established analytical techniques to link phytochemical composition with pharmacological activity is still lacking, despite the mounting body of evidence. Thus, the goal of this study is to thoroughly screen Artemisia pallens extracts for phytochemicals while also assessing a few pharmacological characteristics, like antioxidant and antimicrobial activity. This will not only improve the plant's phytochemical profile but also bolster its therapeutic value in contemporary herbal medicine.

Material and method

Plant material

Natural biomass

Artemisia pallens Wall. Seeds and whole fresh plant material was collected from farmers of Davanemala near Jejuri of Pune district situated at latitude 18.292406^0 and 74.142209^0 longitude. Plant material was dried under shade. Coarsely powdered plant material used for analysis.'

Extraction method

The coarsely powdered whole plant material of *Artemisia pallens* were extracted with different solvents like, chloroform, 70% ethanol, methanol, petroleum ether and distilled water. 10gm powder of *Artemisia pallens* was dissolved in 200 ml of solvent and put on a rotary shaker for 24 hours. Then filtered through muslin cloth and then through Whatman filter paper twice and dried to prepare extract. The dry extract was dissolved in a respective solvent and made mg ml^{-1} solution for further analysis.

Phytochemical activities

Preliminary analysis

Qualitative analysis for all extracts of *Artemisia pallens* was done by methods described in Wagay *et al*., 2017; Kokate, 2005; and Harborne, 1973. Phytochemical analysis for secondary metabolites like alkaloid, phenols, tannins, steroids, terpenoids, flavonoids, glycosides and carbohydrates were carried out by standard method. For all tests mg ml^{-1} solution of different solvents were used.

Quantitative analysis

Estimation of total phenol content (TPC)

Total phenol content was estimated by using Suresh *et al*., (2012) [14] method. Absorbance of the mixture was recorded at 750 nm, using Shimadzu 1800 UV-Vis spectrophotometer (Shimadzu Corporation, Japan) against the blank containing 0.1 ml of extraction solvent. The amount of gallic acid was calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid standard solution (concentration range 10-100 μgml^{-1}) and expressed as mg of Gallic Acid equivalent gm^{-1} dry weight (DW) of the plant material. The total phenolic content in the plant extract was calculated using the formula:

Total phenolic content = GAE ×df × V/m OR TPC =X × df × V/m

Where GAE is the gallic acid equivalence (mg/ml) or concentration of gallic acid established from the calibration curve (Y = 0.0018x; & R^2 = 0.9928); V is the volume of extract in ml and m is the mass (g) of the pure plant extract. X is the concentration value obtained from the standard calibration curve. The data were presented as the average of triplicate analyses.

Estimation of total flavonoid content (TFC)

Total flavonoid content was determined by the Aluminium chloride colorimetric method as described by Zia Zhishen *et al*., (1999) [18], Kim *et al*., (2013a) [6] with some minor modifications; Pala *et al*., (2016a) [10]. To estimate flavonoids, a colorimetric approach using Aluminium chloride was employed(Ahmed, 2018) [1].

Absorbance of the reaction mixture was measured at 510 nm with a Shimadzu 1800 UV - Vis spectrophotometer (Shimadzu Corporation, Japan). Results were expressed as rutin equivalent per gram of dry weight mg RE. The total flavonoid content in the plant extract was calculated using the formula:

Total flavonoids content = X × df ×V/m

Where RE is the rutin equivalence (mg ml^{-1}) or concentration of rutin established from the calibration curve (Y=0.0011x=0.0617, R^2 = 0.9984)

V is the volume of extract in mL and m is the mass (g) of the pure plant extract.

The data were presented as the average of triplicate analyses.

Total Tannic acid content (TTC)

The total tannic acid content of *Artemisia pallens* was calculated by using Veena Sharma and Astha Agarwal (2015) suggested Folin-Ciocalteu assay with little modification (Suresh *et al*., 2011) [17]. The amount of tannic acid was expressed as mg tannic acid equivalent (TAE) per gm of plant dry powder. Total Tannic acid content was calculated using formula as:

Total tannic acid content (TTC) = X × df ×V/m OR TAE × df ×V/m

The data were presented as the average of triplicate analyses.

Pharmacological analysis

Antioxidant activity of *Artemisia pallens* Wall. By DPPH method

DPPH antioxidant assay was carried out as described by Duh *et al*. (2001), Oyaizu, (1986) and Suresh *et al*., (2011) [17]. The assay was carried out using Shimadzu 1800 UV-VIS spectrophotometer (Shimadzu corporation). Absorbance of each solution was measured at 517 nm against 0.3 mM DPPH. IC_{50} values calculated denote the concentration of a sample required to decrease the absorbance by 50%. % scavenging activity was calculated by using formula:

$$\% \text{ scavenging} = \frac{A_0 - A_s}{A_0} \times 100$$

IC_{50} value was calculated by using equation y

$$= 1.0002x + 3.1967$$

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is based on the generation of hydroxyl radicals from a Fenton reaction between ferrous ions and hydrogen peroxide of the reaction mixture. The hydroxyl radicals formed by the Fenton reaction react with deoxyribose to form malonaldehyde which gives a pink chromogen when heated with thiobarbituric acid at low pH conditions. The ability to scavenge hydroxyl radicals is measured by the ability of extracts to prevent the degradation of deoxyribose by the hydroxyl radicals generated in the reaction mixture.

The IC_{50} to scavenge the hydroxyl radicals for the methanolic extract of plant and standard were 3855.49µg/gm dry weight of plant extract and 278.72 µg/gm of gallic acid respectively.

Antimicrobial activity

Antimicrobial activity was performed by using cup plate method as described by Suresh *et al*., 2011; (Gajjar *et al*., 2019 [4]; Kanimozhi1 & Balaji2, 2018 [5]; Suresh *et al*., 2011) [17]. Two Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis)* & Gram-negative bacteria (*Pseudomonas species, Escherichia coli)* were used for antibacterial activity. Streptomycin was used as standard, which was dissolved in dimethyl sulphoxide (DMSO). Ethanolic (95%), methanolic, aqueous and petroleum ether Plant extracts were prepared using cold maceration method. Bacteria were grown in

Nutrient broth media antimicrobial activity was performed by using Nutrient agar media (pH = 7.2 – 7.6).

Microorganism preparation

Pure cultures of *E. coli*, *Pseudomonas* sp., *Bacillus subtilis* and *B. cereus* were procured and maintained on recommended medium.

Screening of Antibacterial activity

The antibacterial activity of different plant extracts of *Artemisia pallens* was evaluated by cup plate method using different dilutions *viz*., 1 mg ml^{-1}, 2 mg ml^{-1}, 3mg ml^{-1}, 4 mg ml^{-1}, 5mg ml^{-1}, 6mg ml^{-1}. Six mm holes were made on Sterilized nutrient agar plates. 0.1 ml of the test organisms was spread on agar plates. Samples, standard drugs, and solvent control (respective solvents) were added into each hole separately. The plates were maintained at 4º C for 1 h to allow the diffusion of solution into the agar medium. The plates were incubated at 37ºC for bacteria for 24 hours. After 24 hours the zone of inhibition was measured.

Statistical analysis

All experiments were performed in triplicates and data were expressed as mean ± dtandard error (SE). statistical analyses were carried out using IBM SPSS Statistics 20. One-way analysis of variance (ANOVA) was used to determine significant differences among solvent systems, followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

Results

Phytochemical analysis

Table 1: Qualitative phytochemical analysis of *Artemisia pallens* Wall.

Sr. No.	Name of Test	Methanol	chloroform	Petroleum ether	Ethanol	Distilled water
Alkaloids						
a.	Mayers Test	+	-	+	-	-
b.	Wagners Test	+	-	+	-	-
Phenols						
a.	Lead acetate Test	+	+	-	+	-
b.	Ferric chloride test	-	+	-	+++	-
Steroids						
a.	Salkowski Test	+	+	-	+++	+
Tannin						
a.	Lead acetate Test	+	+	+	+	+
b.	Gelatin test	+	+	+	-	-
Saponin						
a.	Foam test					
Flavonoids						
a.	Alkaline reagent Test	-	+	-	+++	++
Terpenoids						
a.	Salkowaski test	+++	-	+	-	+
Glycosides						
a.	Modified Borntragers test	+	+	+++	+++	++
Proteins						
a.	Biuret test	-	+	+	-	-

+ Sign indicates presence of test, while – sign indicates absence of test

Alkaloids are present in methanol and petroleum ether extract and absent in chloroform, ethanol and distilled water extract. Phenols are present in methanol, ethanol and chloroform, while absent in petroleum ether and distilled water. Steroids are present in all extracts except in petroleum ether. Tannin is present in all extract (for gelatin test absent in ethanol and distilled water extract). Flavonoids are present in chloroform, ethanol and distilled water extract and absent in methanol and petroleum ether. Terpenoids are present abundantly in methanol extract and absent in chloroform and ethanol extract. Glycosides are present in all extracts but abundant in petroleum ether and ethanol extract. Proteins are present only in chloroform and petroleum ether extracts.

Quantitative analysis

Total phenol content in Artemisia pallens

The total phenol content present in *A. Pallens* was estimated by using modified assay of Ozsoy *et al*. The total phenol content is reported as gallic acid equivalents by reference to standard curve ($Y = 0.0022x + 0.085$; $R^2 = 0.9978$). One-way ANOVA showed a significant difference in total phenolic content (TPC) among the different solvent extracts, F (4, 10) = 789.888, P = 0.000. Tukey's post hoc test revealed that distilled water (25.40 ± 0.45^{d}) had significantly high phenolic content than ethanolic (9.99 ± 0.41c), methanolic (10.02 ± 0.13^{c}), petroleum ether (2.40 ± 0.04^{b}) and chloroform extract (0.35 ± 0.03^{a}) at $p < 0.05$. ethanolic and methanolic extracts were not significantly different ($p < 0.05$).

Table 2: Quantitative analysis of Total Phenol, Flavonoid and Tannin content in natural biomass using different solvent system

Extraction solvent	Total Phenol content (GAE mg/ gm dry extract)	Total Flavonoid content (RE mg/ gm dry extract)	Total Tannin content (TAE mg/ gm dry eextract)	Artemisinin content (ARTE mg/ gm dry extract)
Distilled Water	25.40 ± 0.45^{d}	120.34 ± 0.03^{ab}	5.61 ± 0.98^{a}	0.12 ± 0.00^{b}
Ethanol	9.99 ± 0.41^{c}	121.03 ± 0.05^{c}	12.75 ± 1.94^{b}	1.01 ± 0.04^{d}
Methanol	10.02 ± 0.13^{c}	122.10 ± 0.12^{d}	20.98 ± 2.07^{c}	0.95 ± 0.10^{c}
Petroleum Ether	2.40 ± 0.04^{b}	120.47 ± 0.03^{b}	-	0.09 ± 0.02^{a}
Chloroform	0.35 ± 0.03^{d}	120.13 ± 0.06^{a}	-	0.28 ± 0.00^{ab}

Values are expressed as mean ± SE (n = 3). Different superscript letters (a – d) indicate significant differences at $p < 0.05$ (Tukey's HSD).

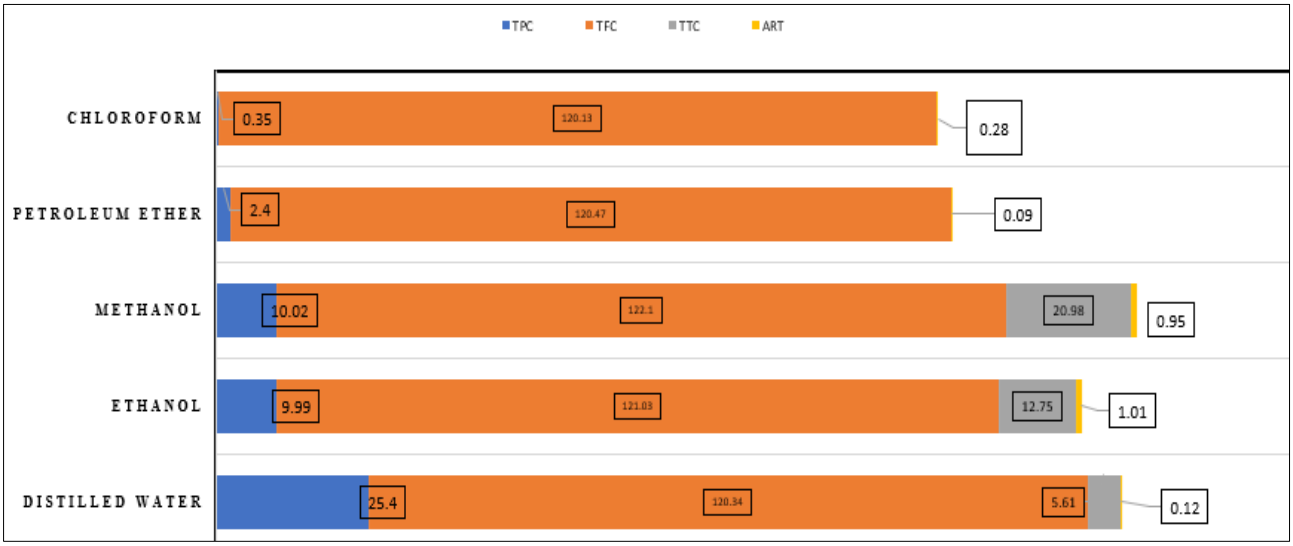

Fig 1: Correlation between total phenol flavonoid, tannin and artemisinin content

Total tannin content in *Artemisia pallens* Wall.

Total tannic acid content present in Artemisia pallens is reported as tannic acid equivalents by reference to standard curve (Y = 0.0022x + 0.0779; R^2 = 0.9991). The comparison of Total tannic acid content among the three solvent extracts showed statistically significant differences. Methanolic extract exhibited the highest tannin content (20.98 ± 2.07^c) which was significantly greater than that of ethanolic (12.75 ± 1.94^b) and distilled water (5.61 ± 0.98^a) extracts according to Tukey's HSD test at $p < 0.05$.

Estimation of total flavonoids content

Total flavonoid content present in *Artemisia pallens* was reported as rutin equivalents according to standard graph (Y = 2.2246x – 0.0131 and R^2 = 0.9903). total flavonoid content was significantly high in ethanolic (121.03 ± 0.05^c) and methanolic (122.10 ± 0.12^d) extracts. Distilled water extract (120.34 ± 0.03^{ab}), petroleum ether extract (120.47 ±0.03^b) and chloroform extract (120.13 ± 0.06^a) did not showed significant difference in flavonoid content ($p < 0.05$).

Quantification of Artemisinin (ART)

Artemisinin content was quantified by using spectrophotometric method and reported as standard artemisinin equivalents according to standard graph y = 0.0003 + 0.0633 & R^2= 0.9229. The comparison among five solvent extracts showed statistically significant differences ($p < 0.05$). Ethanolic extract indicated the highest artemisinin content (1.01 ± 0.04^d), which was significantly greater than methanolic (0.95 ± 0.10^c), distilled water (0.12 ± 0.00^b), and chloroform (0.28 ± 0.00^{ab}) extracts. Petroleum ether extract indicated very less amount of artemisinin content (0.09 ± 0.02^a).

Pharmacological analysis

Antioxidant activity by DPPH Method

The plant contains ascorbic acid and phenolics both of which are powerful antioxidants. Increased antioxidant activity correlated with the high flavonoids content (Matvieieva *et al*., 2019; Ali *et al*., 2013) [2] Utilizing the 2,2-diphenyl, 1-picryl hydrazyl (DPPH) method with ascorbic acid as a reference, the methanol extract of the aerial portions of *Artemisia pallens* Wall was evaluated for its antioxidant activity due to its phenolic and flavonoid contents utilizing radical scavenging assays (Ruikar *et al*., 2011) [13].

Artemisia pallens indicated high antioxidant activity (IC_{50} = 177.83 µgml^{-1} of DPPH) due to high flavonoid content present in the plant.

Table 3: Antioxidant activity of *Artemisia pallens* by DPPH method

Concentration of plant sample µgml^{-1}	% Free radical scavenging of Artemisia pallens	Concentration of Ascorbic acid in µgml^{-1}	% Free radical scavenging of Ascorbic acid
2	0.71 ± 0.21	1	4.72 ± 0.24
4	2.14 ± 0.10	2	5.74 ± 0.19
8	2.14 ± 0.31	5	7.09 ± 0.25
16	2.5 ± 0.24	10	10.76 ± 1.60
32	5.0 ± 0.25	15	18.01 ± 0.20
64	6.7 ± 0.18	25	29.49 ± 0.18
128	7.14 ± 0.19	50	52.84 ± 0.17
256	13.57 ± 0.20	-	-
512	19.28 ± 0.22	-	-
1000	49.64 ± 0.19	-	-
IC_{50}	177.83 µgml^{-1} of DPPH	-	15.6 µgml^{-1} of DPPH

Values are expressed as mean ± SE (n = 3).

Antioxidant activity by Radical Scavenging Assay

Phenolic compounds are responsible for the antioxidant activity of the plants. Hydroxyl radical scavenging activity of whole plant methanolic extract was quantified by hydroxyl radical scavenging method as described by Suresh *et al*., 2011 [17]. Whole plant extract of *A. pallens* indicates very low radical scavenging activity (IC_{50}= 3855.49 µg ml^{-1} dry plant extract) as compared to the standard (IC_{50}=278.72 µg ml^{-1} of gallic acid).

Table 1: Antioxidant activity of *Artemisia pallens* by Hydroxyl Radical Scavenging method

Concentration of plant sample in µg ml^{-1}	% Free radical scavenging of Artemisia pallens	Concentration of Ascorbic acid in µg ml^{-1}	% Free radical scavenging of Ascorbic acid
50	4.32	2	3.37
100	5.27	4	3.78
150	5.4	6	3.91
200	5.94	8	4.45
1000	15.81	10	4.72
IC50	3855.49 µg ml^{-1} dry plant extract	IC50	278.72 µg ml^{-1} of gallic acid

Values are expressed as mean ± SE (n = 3).

Antimicrobial activity

Antimicrobial activity of *Artemisia pallens* was performed against two gram-negative bacteria, P*seudomonas sps.* and *E. coli* and two gram-positive bacteria namely *Bacillus subtilis and Bacillus cereus* using Streptomycin as standard drug by agar diffusion method. Statistical analysis was carried out by ANOVA test ($P \leq 0.05$) and Ducan's Multiple Range Test (DMRT) post hoc test. Results were as shown in Table No. 5 to Table No. 8.

Antimicrobial activity of *E. coli*

Ethanolic extract of *Artemisia pallens* (11.33 ± 1.15 mm) showed best antimicrobial activity against *E. coli* as compared to methanolic extract (9.0 ± 0.57 mm). Petroleum ether extract (6.75 ± 1.81 mm) showed very poor antimicrobial activity. But antimicrobial activity of all three extract was significantly low as compared to standard drug streptomycin (14.3 ± 0.88 mm at $P \leq 0.05$).

Table 5: Antimicrobial activity of *E. coli*

Plant Extract Concentration (mg ml^{-1})	Zone of Inhibition (mm) for		
	Methanol (Mean ± SE)	Ethanol (Mean ± SE)	Petroleum ether (Mean ± SE)
1	3.0 ± 1.00^{a}	1.33 ± 0.57^{a}	3.08 ± 0.58^{a}
2	6.0 ± 0.88^{ab}	3.00 ± 0.66^{a}	3.86 ± 0.86^{a}
3	6.3 ± 0.88^{bc}	3.67 ± 0.88^{a}	3.92 ± 0.72^{a}
4	8.0 ± 1.00^{bc}	4.33 ± 0.57^{a}	3.54 ± 0.74^{a}
5	9.0 ± 0.57^{c}	10.67 ± 0.88^{b}	2.98 ± 0.91^{a}
6	8.7 ± 0.66^{c}	11.33 ± 1.15^{b}	2.68 ± 0.91^{a}
Streptomycin (Standard)	14.3 ± 0.88^{d}	14.3 ± 0.88^{c}	14.3 ± 0.88^{b}

Values are expressed as mean ± SE (n = 5). Different superscript letters (a – d) indicate significant differences at $p < 0.05$ (Tukey's HSD).

Antimicrobial activity of *Pseudomonas* sps.

For methanolic and ethanolic extract antimicrobial activity was increased with increase in concentration. Maximum zone of inhibition was observed 2.83 ± 0.91mm and 4.67 ± 1.00 mm for methanolic and ethanolic extracts respectively. For petroleum ether extract maximum zone of inhibition was observed at 3 mgml^{-1} (6.75 ± 1.81mm) concentration after that zone of inhibition was decreased with increase in concentration of plant extract. For all three plant extracts antimicrobial activity was significantly low as compared to standard drug streptomycin (19.28 ± 2.38 mm).

Table 6: Antimicrobial activity of *Pseudomonas* sps.

Plant extract concentration (mgml^{-1})	Zone of Inhibition (mm) for		
	Methanol (Mean ± SE)	Ethanol (Mean ± SE)	Petroleum ether (Mean ± SE)
1	0.92 ± 0.42^{a}	1.11 ± 0.40^{a}	3.17 ± 1.23^{a}
2	1.5 ± 0.25^{a}	2.78 ± 0.77^{a}	5.00 ± 1.51^{a}
3	1.75 ± 0.59^{a}	2.89 ± 0.55^{a}	6.75 ± 1.81^{a}
4	1.83 ± 0.71^{a}	3.56 ± 0.59^{a}	5.08 ± 1.48^{a}
5	2.17 ± 0.84^{a}	4.67 ± 0.51^{a}	4.83 ± 1.23^{a}
6	2.83 ± 0.91^{a}	4.67 ± 1.00^{a}	2.5 ± 0.19^{a}
Streptomycin (Standard)	19.28 ± 2.38^{b}	19.28 ± 2.38^{b}	19.28 ± 2.38^{b}

Values are expressed as mean ± SE (n = 5). Different superscript letters (a – d) indicate significant differences at $p < 0.05$ (Tukey's HSD).

Antimicrobial activity of *Bacillus subtilis*

Methanolic, ethanolic as well as petroleum ether extract of *Artemisia pallens* showed best antimicrobial activity against *Bacillus subtilis*. As concentration increases zone of inhibition was increased for all three extracts except 6 mgml^{-1} petroleum ether extract (11.73 ± 0.46 mm) (zone of inhibition decreased as compared to 5 mgml^{-1} extract 13.4 ± 0.43 mm). For methanolic plant extract of 4 mgml^{-1}, 5 mgml^{-1} and 6 mgml^{-1} zone of inhibition (8.8 ± 0.91 mm, 10.07 ± 0.38 mm and 12.53 ± 0.96 mm respectively) was significantly high as compared to standard drug streptomycin (5.10 ± 0.17 mm).

Ethanolic extracts also indicates significant increase in antimicrobial activity for 4 mgml^{-1}, 5 mgml^{-1} and 6 mgml^{-1} (zone of inhibition were 8.8 ± 0.43mm, 10 ± 0.37 mm and 10 ± 0.37 mm respectively) as compared to standard drug streptomycin (5.10 ± 0.17 mm).

Petroleum ether extract showed best result as compared to methanolic and ethanolic extract. Petroleum ether plant extract from 3 mgml^{-1} to 6 mgml^{-1} concentration showed significantly increase in antimicrobial activity as compared to standard drug streptomycin (5.10 ± 0.17 mm).

Table 7: Antimicrobial activity of *Bacillus subtilis*

Plant extract concentration (mgml^{-1})	Zone of Inhibition (mm) for		
	Methanol	Ethanol	Petroleum ether
1	4.23 ± 0.72^{a}	3.77 ± 0.27^{a}	4.27 ± 0.41^{a}
2	6.13 ± 0.24^{ab}	5.17 ± 0.20^{b}	4.9 ± 0.32^{ab}
3	6.83 ± 0.97^{bc}	7.5 ± 0.07^{c}	5.97 ± 0.28^{bc}
4	8.8 ± 0.91^{cd}	8.8 ± 0.43^{d}	7.17 ± 0.77^{c}
5	10.07 ± 0.38^{d}	10 ± 0.37^{e}	13.4 ± 0.43^{e}
6	12.53 ± 0.96^{e}	12.03 ± 0.53^{f}	11.73 ± 0.46^{d}
Streptomycin (Standard)	5.10 ± 0.17^{ab}	5.10 ± 0.17^{c}	5.10 ± 0.17^{ab}

Values are expressed as mean ± SE (n = 5). Different superscript letters (a – d) indicate significant differences at $p < 0.05$ (Tukey's HSD).

Antimicrobial activity of *Bacillus cereus*

Methanolic extract showed significantly low antimicrobial activity against *Bacillus cereus* as compared to standard drug except 6 mgml^{-1} concentration (same as standard drug). Ethanolic extract indicated significantly increase in antimicrobial activity from 3mgml^{-1} and above concentration as compared to streptomycin (5.03 ± 0.14 mm) as shown in Table No.8 Petroleum ether extract shown same result like methanolic extract.

Table 8: Antimicrobial activity of *Bacillus cereus*

Plant extract concentration (mgml^{-1})	Zone of Inhibition (mm) for		
	Methanol (Mean ± SE)	Ethanol (Mean ± SE)	Petroleum ether (Mean ± SE)
1	NR	3.07 ± 0.58^{a}	2.13 ± 0.22^{a}
2	1.5 ± 0.80^{a}	4.23 ± 0.32^{ab}	2.17 ± 0.35^{a}
3	1.7 ± 0.65^{a}	7.1 ± 0.11^{c}	2.87 ± 0.27^{a}
4	2.4 ± 0.66^{a}	7.9 ± 0.55^{cd}	2.93 ± 0.63^{a}
5	3.3 ± 0.75^{ab}	9.4 ± 0.43^{de}	4.13 ± 0.23^{b}
6	4.87 ± 0.99^{b}	10.47 ± 0.93^{e}	5.2 ± 0.11^{c}
Streptomycin (Standard)	5.03 ± 0.14^{b}	5.03 ± 0.14^{b}	5.03 ± 0.14^{bc}

Values are expressed as mean ± SE (n = 5). Different superscript letters (a – d) indicate significant differences at $p < 0.05$ (Tukey's HSD).

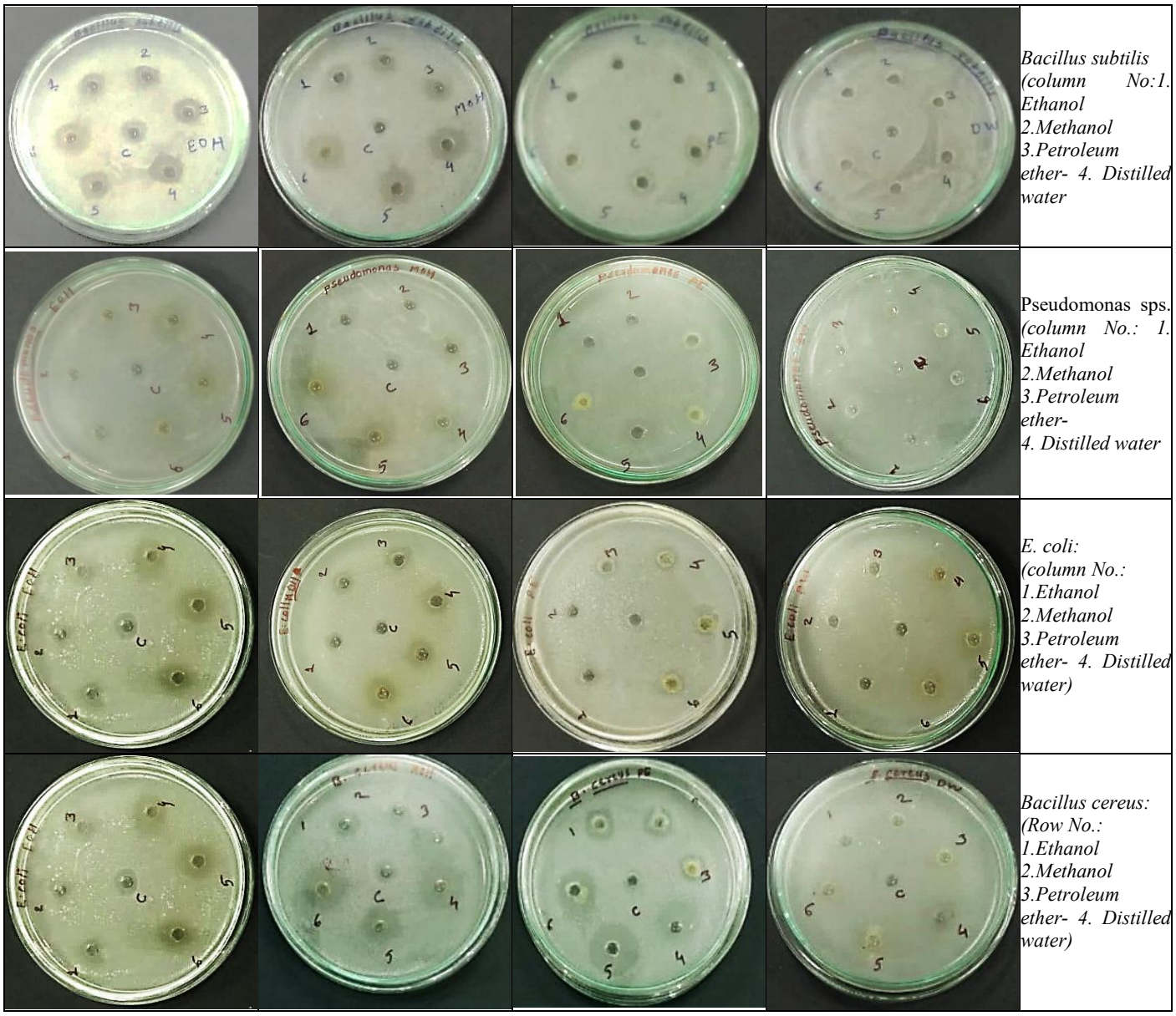

Fig 2: Antimicrobial activity *of B. cereus, Bacillus subtilis, E. coli and Pseudomonas sps*. For 1 – 6 mg/ml Plant extract of methanol, Ethanol, Petroleum ether, Distilled water and streptomycine as control

Result indicates that all three extracts (Methanolic, Ethanolic and Petroleum ether) showed best antimicrobial activity against *Bacillus subtilis*, while antibacterial activity against *E. coli* and *Pseudomonas* sps., was significantly low as compared to standard drug streptomycin. Only Ethanolic extract showed best antimicrobial activity against *Bacillus cereus*.

Discussion

The phytochemical content of plants fluctuates according to changes in climate, environmental factors, soil composition, plant portions utilized for solvent system analysis, extraction technique, etc. Flavonoid content varies with change in climate and environmental conditions(Kim *et al*., 2013b) [7]. Total phenol, flavonoid and terpenoid content for leaf and bud acetone extract was 275.5 ± 0.01µg/ml, 9.1 ± 0.004 µg ml^{-1} and 68.5 ± 0.01µg ml^{-1} respectively(Kulkarni & Kamble, 2022) [8]. Total flavonoid, phenol, and tannin content in *In-vitro* grown aerial parts of *Artemisia* pallens was maximum in methanolic(3.8 ± 0.01mg RE /gm DE, 3.7±0.01 TAE mg/gm DE and 3.7 ± 0.1 TAE mg /gm DE) respectively (Pala *et al*., 2016b) [11]. *Artemisia pallens* aerial plant parts collected froNilgiri hills showed high amount of total phenol and flavonoid content as compared to others for ethanolic extracts, 6.88 ± 0.052 mg GAE/gm DW and 11.55 ± 0.006 mg RE /gm DW respectively.

Recent study showed that total phenol, flavonoid and tannin content was high in distilled water extract (25.40 ± 0.45^{d} mg GAE/gm DW), methanolic extract (122.10 ± 0.12^{d} mg RE/gm DW) and 20.98 ± 2.07^{e} mg TAE/gm DW of total phenol, flavonoid and Tannin content respectively for naturally grown plant material collected from Jejuri. Total phenol, flavonoid and tannin content quantified in present study is significantly high as compared to the previous study. So cold extraction method is best for extraction of all three phytochemicals.

Ethanolic, 95% ethanolic and methanolic extract of *Artemisia pallens* has IC_{50} value 3.013µgml^{-1}, 150.33±1.05µgml^{-1} and 292.7 µgml^{-1} respectively (Devare *et al*.,2013., Ruikar *et al*., 2011 [13] and J. Suresh *et al*., 2011) [17] for root, and aerial parts respectively. So, it indicates that root has best antioxidant activity as compared to aerial parts. In present study whole plant biomass antioxidant activity was measured by DPPH assay (IC_{50} =177.83 ± 0.13 µg ml^{-1} dry plant

extract) and hydroxyl radical scavenging assay (IC_{50} =3855.49 µg ml^{-1} dry plant extract)

According to previous research *Artemisia pallens* did not shown antibacterial activity at 10, 20 and 30 $mgml^{-1}$ ethanolic extract concentration against Bacillus subtilis. But according to recent study ethanolic extract showed antibacterial activity against Bacillus subtilis. Ethanolic extract showed significant high antimicrobial activity against *Bacillus subtilis* and *Bacillus cereus*. While methanolic and petroleum ether extract showed significantly high antibacterial activity against *Bacillus subtilis*. This study showed that ethanolic extract showed best antimicrobial activity against *E. coli. Pseudomonas sps., Bacillus subtilis and Bacillus cereus*. Methanolic extract showed best result against E. coli and Bacillus subtilis. While petroleum ether was best for *Pseudomona*s as compared to methanolic and ethanolic extract.

Conclusion

The present study demonstrated that *Artemisia pallens* is a medicinally valuable plant enriched with diverse phytochemicals, particularly phenols, flavonoids, tannin and artemisinin. extraction efficiency and phytochemical yield were strongly influenced by the choice of solvent system, with best solvent (methanol) for flavonoid and tannin and ethanol for artemisinin providing maximum recovery. A positive correlation between phytochemical content, artemisinin concentration and antioxidant activity were observed, indicating that secondary metabolite contributes significantly to the pharmacological potential of *A. pallens*. These findings suggest that optimization of solvent extraction and selection of suitable plant parts can enhance therapeutic applications, supporting the use of *A. pallens* as promising source of natural antioxidants and bioactiv compounds for drug development.

Acknowledgement

Author is thankful to the Chhatrapati Shahu research, training and human development institute (Sarthi), Pune (An Autonomous Institute of the Government of Maharashtra) for financial support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ahmed F. Antioxidant activity of Ricinus Communis. Organic & Medicinal Chemistry International Journal. 2018;5(4). https://doi.org/10.19080/omcij.2018.05.555667
2. Ali M, Abbasi BH, Ihsan-ul-haq. Production of commercially important secondary metabolites and antioxidant activity in cell suspension cultures of *Artemisia absinthium* L. Industrial Crops and Products. 2013;49:400-406. https://doi.org/10.1016/j.indcrop.2013.05.033
3. Bail S, Buchbauer G, Schmidt E, Wanner J, Slavchev A, Stoyanova A, *et al*. GC-MS-analysis, antimicrobial activities and olfactory evaluation of essential davana (*Artemisia pallens* Wall. ex DC) oil from India. Natural Product Communications. 2008;3(7). https://doi.org/10.1177/1934578x0800300705
4. Gajjar P, Deshpande R, Kukreja T. Study of evaluation of antimicrobial property of different concentrations of *Artemisia pallens* (Davana) extract against Streptococcus mutans serotype c (ATCC 25175). Indian Journal of Basic and Applied Medical Research. 2019;8:154-157. www.ijbamr.com
5. Kanimozhi NV, Balaji N. An *In vitro* study of Anti-Inflammatory, Antioxidant and Antimicrobial Potential of *Artemisia pallens*. International Journal of Modern Science and Technology. 2018;3(7):161-165. https://www.researchgate.net/publication/364780354
6. Kim YJ, Lee JH, Kim SJ. Cultivation characteristics and flavonoid contents of wormwood (*Artemisia montana* Pamp.). Journal of Agricultural Chemistry and Environment. 2013a;2(4):117-122. https://doi.org/10.4236/jacen.2013.24017
7. Kim YJ, Lee JH, Kim SJ. Cultivation characteristics and flavonoid contents of wormwood (*Artemisia montana* Pamp.). Journal of Agricultural Chemistry and Environment. 2013b;2(4):117-122. https://doi.org/10.4236/jacen.2013.24017
8. Kulkarni AA, Kamble RP. α-Amylase Inhibitory Secondary Metabolites from *Artemisia pallens* Wall ex DC—Biochemical and Docking Studies. 2022;73. https://doi.org/10.3390/iecps2021-11978
9. Kumar AP, Kumud U. Pharmacognostic and phytochemical investigation of aerial parts of *Artemisia pallens* Wall ex.Dc. Pharmacognosy Journal. 2010;2(9). https://doi.org/10.1016/S0975-3575(10)80117-8
10. Pala Z, Shukla V, Alok A, Kudale S, Desai N. Enhanced production of an anti-malarial compound artesunate by hairy root cultures and phytochemical analysis of *Artemisia pallens* Wall. 3 Biotech. 2016a;6(2). https://doi.org/10.1007/s13205-016-0496-5
11. Pala Z, Shukla V, Alok A, Kudale S, Desai N. Enhanced production of an anti-malarial compound artesunate by hairy root cultures and phytochemical analysis of *Artemisia pallens* Wall. 3 Biotech. 2016b;6(2). https://doi.org/10.1007/s13205-016-0496-5
12. Pavithra KS, Annadurai J, Ragunathan R. *In vivo* Antidiabetic Property of *Artemisia Pallens* Using Albino Rats as Model. International Journal of Pharmacy and Biological Sciences-IJPBS TM. 2020;2:10. https://doi.org/10.21276/ijpbs.2020.10.2.19
13. Ruikar A, Khatiwora E, Ghayal N, Misar A, Mujumdar A, Puranik V, *et al*. Studies on aerial parts of *Artemisia pallens* wall for phenol, flavonoid and evaluation of antioxidant activity. Journal of Pharmacy and Bioallied Sciences. 2011;3(2):302-305. https://doi.org/10.4103/0975-7406.80768
14. Suresh J, Ahuja J, Paramakrishnan N, Sebastian M. Total Phenolic and Total Flavonoids Content of Aerial Parts of *Artemisia abrotanum* Linn. and *A. pallens* Wall. Analytical Chemistry Letters. 2012;2(3):186-191. https://doi.org/10.1080/22297928.2000.10648268
15. Suresh J, Singh A, Vasavi A, Ihsanullah M, Mary S. Phytochemical and pharmacological properties of *Artemisia Pallens*. ijpsr. 2011a;2(12):3081-3090. www.ijpsr.com
16. Suresh J, singh A, Vasavi A, Ihsanullah M, Mary S. phytochemical and pharmacological properties of *artemisia pallens*. ijpsr. 2011b;2(12):3081-3090. www.ijpsr.com
17. Suresh J, Suresh J, Vasavi Reddy SA, Sebastian M, Rajan SK, Author C, *et al*. antioxidant and antimicrobial Activity of *Artemisia pallens*. In: S Journal of

Pharmacognosy and Herbal Formulations J. Suresh *et al*. 2011;1(2). www.ijpijournals.com
18. Zia Zhishen, Tang Mengcheng, Wu Jianming. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. 1999;555-559.