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Research Article

An Invitro study of Anti-Inflammatory, Antioxidant and Antimicrobial Potential of *Artemisia pallens*

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Abstract

Artemisia pallens, commonly known as Davana, is an aromatic herb found abundantly in humid habitats in the plains all over India. The plant is accredited with anthelmintic, antipyretic and tonic properties and also considered as a good fodder. Phytochemical screening of this herb showed the presence of various phytoconstituents. Extracts were analyzed for their antimicrobial capacity against bacterial strains and fungal strains and their zone of inhibition was calculated. It was done by well diffusion method. Also anti-inflammatory activity was verified i.e., protein denaturation technique is used to evaluate the anti-inflammatory activity of the extract. Finally antioxidant capacity of the ethanol extract of *Artemisia pallens* was investigated by phosphomolybdenum method. The extract showed strong antioxidant activities indicating that *Artemisia pallens* extract functions an efficient antioxidant to scavenge free radicals and reduces free radical induced cellular damage.

Keywords: *Artemisia pallens*; Antimicrobial; Anti-inflammatory; Protein denaturation; Phosphomolybdenum method.

Introduction

Artemisia pallens is a small and aromatic herbaceous plant which is native to the southern part of India, especially to the states of Karnataka, Tamilnadu, Andhra Pradesh and in Maharashtra. Genus *Artemisia* popularly known as "Sage Brush" or "Worm wood" is bitter aromatics [1].

Artemisia pallens is utilized in traditional ayurvedic medicinal formulations. Essential oil of davana is useful as antiseptic and disinfectant. Oral administration of methanol extract of this extract led to significant blood glucose lowering effect in glucose fed hyperglycemic and alloxan induced diabetic rats studied [2].

Artemisia pallens commonly known as "Davana" has been traditionally used in Indian folk medicine for the treatment of diabetes mellitus, wound healing and immunomodulating, antihelmintic, antipyretic, antibacterial, antifungal, tonic properties and stimulant. It is also considered as good fodder [3].

In current study the anti-inflammatory activity was investigated by Protein denaturation technique. There were no reports about the antioxidant and antimicrobial activity of stem extract of *Artemisia pallens*. Hence, in the present study, the ethanolic extract of *Artemisia pallens was* screened for antioxidant and antimicrobial activities.

Materials and methods

Chemicals used

Ethanol, Ethyl acetate, Petroleum ether, Ferric chloride, Sodium hydroxide, Sulphuric acid, Fehling's solution A, B, Hydrochoric acid, Meyer's reagent, Olive oil, ǎ naphthol, Benedicts reagent, Ninhydrin, Tetracycline, Ammonium molybdate, Sodium phosphate, Sulphuric acid, Egg albumin, and Phosphate buffer.

Instruments/apparatus required

Magnetic stirrer, Weighing balance, Heating mantle, Aluminium foil, Petri plates, Micro pipettes, and Colorimeter.

Test organisms used

Bacillus subtilis, Escherichia Coli, Klebsiella pneumoniae, Staphylococcus aureus, Trichoderma sp., and *Candida albicans.*

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Preparation of plant extract

Magnetic stirrer method of extraction was carried out to prepare the crude extract of *Artemisia pallens* stem. Powdered plant material (10 g) was taken in the beaker and extracted with organic solvents (200 ml) such as ethanol, ethyl acetate and petroleum ether. They were left for 2 days in the magnetic stirrer [4]. This procedure was repeated four to five times and then the resulting infusion was filtered using a normal filter paper. The yield percent was then calculated using the liquid extract obtained after filtration.

Phytochemical analysis

Detection of protein

Ninhydrin test: 1 ml of the extract was mixed with 1ml of 0.2% of ninhydrin solution. The mixture is added to acetone and heated in a boiling water bath for few minutes till purple colour is obtained indicating the presence of proteins.

Million's test: 1 ml of the extract was mixed with 15% of mercuric sulphate in 15% sulphuric acid and heated in the boiling water bath for ten minutes and then cooled. It is treated with sodium nitrate solution resulting in the presence of red colour indicating the presence of proteins.

Detection of alkaloids

3 ml of the extract was mixed with 1 ml of 1% hydrochloric acid and heated for 20 minutes, cooled. Meyer's reagent is added. Creamy precipitate indicates the presence of alkaloids.

Detection of steroids

5 drops of concentrated sulphuric acid is added along the sides of the test tube to 2 ml of the extract, red color indicate the presence of steroids.

Detection of flavonoids

1 ml of 10% sodium hydroxide was added to 3 ml of the extract. There was yellow coloration which is indicative the presence of flavonoids [5].

Detection of glycosides

5 drops of sulphuric acid was added to 1 ml of the extract and mixture heated in boiling water bath for about 15 min. 3 ml of Fehling's solution was then added and the mixture is boiled. A brick red precipitate was confirmatory for the presence of glycosides [6].

Detection of saponins

Frothing test: 2 ml of the extract was vigorously shaken in the test tube for 2 minutes. Frothing was observed.

Emulsion test: 5 drops of olive oil was added to 3 ml of the extract in the test tube and vigorously shaken. Presence of stable emulsion formed indicates the presence of saponins.

Detection of phenolics

Two drops of 5% ferric chloride of the extract in a test tube. Presence of greenish precipate indicated the presence of phenolics.

Detection of terpenoids

1 ml of filtrate and 2 ml of chloroform with concentrated sulphuric acid along the sides of the test tube. Presence of reddish brown precipitate indicates terpenoids.

Detection of phlobatannins

2 ml of extract is added into dilute hydrochloric acid. Red precipitate indicates the presence of phlobatannins [7].

Detection of carbohydrates

Molisch's test: 1 ml of the sample with few drops of Molisch reagent (ǎlpha naphthol) and few drops of sulphuric acid along the sides of the test tube. Purple coloured ring at the junction indicates presence of reducing sugar.

Benedict's reagent: 1 ml of sample along with few drops of Benedict's reagent is heated in boiling water bath for few minutes. Reddish orange or brown precipitate indicates the presence of carbohydrates [8].

Invitro studies

Antioxidant assay

The reagent solution is a mixture of sodium phosphate (28 mM), ammonium molybdenate (4 mM) and sulphuric acid (6 M). The sample is taken in five different concentrations (20, 40, 60, 80, 100 µg/mL) along with the solvent (980, 960, 940, 920, 900 µL) respectively [9-12].

1 ml of the reagent solution is added to all the test tubes and kept in a boiling water bath at 95℃ for 90 min. The absorbance was read at

695 nm. Percentage inhibition was calculated using eq. (1) [13].

$$\text{Percentage inhibition} = \frac{(control - sample)}{control} \times 100 \quad (1)$$

The %inhibition was then calculated and 50% inhibitory concentration (IC50) was finally determined.

Antimicrobial activity

Antibacterial activity

The antibacterial activity of the ethanol extract of *Artemisia pallens* was evaluated by well diffusion method using Nutrient Agar medium for the assay. The nutrient agar sterilized and then poured into the petri plate for solidification. Then the microorganisms (*Bacillus subtilis, Esherichia coli, Klebsiella pneumoniae, Staphylococcus aureus)* are traced i.e, swabbed throughout the plate in a uniform manner. For agar well diffusion method, wells were made and the test compound along with an antibiotic(tetracycline) was introduced into separate wells and incubated at 37°C for 24 h [14]. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls (ethanol solvent, DMSO) were maintained.

Antifungal activity

The antifungal activity of the ethanol extract of *Artemisia pallens* was evaluated by well diffusion method using Sabouraud's Dextrose Agar medium for the assay. The SDA was sterilized and then poured into the petri plate for solidification. Then the microorganisms *(Trichoderma sp., Candida albicans)* are traced swabbed throughout the plate in a uniform manner. For agar well diffusion method, wells were made and the test compound was introduced into separate wells and incubated at 37°C for 24 h [14]. Microbial growth was determined by measuring the diameter of zone of inhibition.

Invitro anti-inflammatory assay

Protein denaturation technique

The reaction mixture (0.5 mL; pH 6.3) consisted of 0.45 mL of egg albumin (5% aqueous solution) and 0.05ml of distilled water [15, 16]. pH was adjusted at 6.3 using a small amount of 1 N Hcl. 1000µl *Artemisia pallens* ethanol extract of various concentrations (20, 40, 60, 80, 100 µg/mL) was added to the reaction mixture and were incubated at 37℃ for 30min and then heated at 57℃ for 5 min after cooling the samples, 2.5ml of phosphate buffer was added. Turbidity was measured spectrophotometrically at 600nm. For negative control 0.05ml distilled water and 0.45ml of egg albumin were used. The percentage inhibition of protein denaturation was calculated using eq. (2) [17].

$$\text{Percentage inhibition} = \frac{(control - sample)}{control} \times 100 \quad (2)$$

Results and discussions

Percentage yield of extract

Table 1 represents the yield % of extracts with different solvents. Ethanol extract has higher yield compared to ethyl acetate and petroleum ether extract. Hence the ethanolic extract was used for further analysis.

Table 1. Percentage yield of extract

Solvent used	Yield, (%) w/w
Ethanol	12
Ethyl acetate	7.5
Petroleum Ether	5.2

Preliminary phytochemical analysis

Table 2 represents the presence of phytoconstituets including saponins, proteins, carbohydrates, tannins, terpenoids, phenolics and flavonoids.

Table 2. Phytochemical screening of ethanolic extract of stem of *Artemisia pallens*

S. No.	Phytochemical	Result
1	Saponins Emulsion test	+
2	Steroids	-
3	Proteins-Ninhydrin test	+
4	Million's test	+
5	Carbohydrates-Molisch test	+
6	Benedict's test	+
7	Glycosides	-
8	Taninns	+
9	Phenolics	+
10	Flavanoids	+
11	Alkaloids	-
12	Terpenoids	+

'+' - Present '_' – Absent

Antioxidant activity

Different concentrations from 20-100 µg/mL of the ethanolic extract of stem of

Artemisia pallens were analyzed for antioxidant activity by Phosphomolybdenum method. From the results listed in table 3, ethanolic extract of stem of Artemisia pallens at concentration 100 µg/mL showed highest 94.6 % inhibition. The extract concentration for 50% inhibition (IC50) was determined as 52.7 µg/mL

Table 3. Antioxidant effect of ethanolic extract of stem of *Artemisia pallens*

Concentration (µg/mL)	Absorbance at 680nm	% Inhibition
20	0.36	75
40	0.75	88
60	1.12	91.9
80	1.45	93.7
100	1.76	94.6

Antimicrobial activity of extract

Antibacterial Assay

The zone of inhibition using ethanol extract against different organisms is shown in table 4. The extracts at higher concentrations of 75-100µg/mL showed highest antibacterial activity against both gram positive and gram negative bacterial cultures. Their zone of inhibition was measured and listed in table 4.

Table 4. Antibacterial activity of ethanolic extract of stem of *Artemisia pallens*

Bacteria used	Zone of inhibition (±mm)			
	25 µg/mL	50 µg/mL	75 µg/mL	100 µg/mL
Gram positive				
Bacillus subtilis	5	6	10	10
Staphylococcus aureus	7	6	11	9
Gram negative				
E. coli	8	9	7	11
Klebsiella pneumonia	9	7	8	10

Antifungal Activity

The zone of inhibition using ethanol extract against different organisms is shown in table 5. The ethanolic extract of stem of *Artemisia pallens* showed antifungal activity against both fungal cultures. Their zone of inhibition was measured and listed in table 5. *Candia albicans* showed higher antifungal activity when compared to *Trichoderma sp.*

Table 5. Antifungal activity of ethanolic extract of stem of *Artemisia pallens*

Fungi used	Zone of inhibition (±mm)
Trichoderma sp.	10
Candida albicans	14

Anti-Inflammatory assay

The ability of stem extract to inhibit protein denaturation was investigated to study the mechanism of the anti-inflammation activity. It was observed that maximum inhibition of 82% at 1000 µg/mL was obtained and listed in table 6.

Table 6. Anti-inflammatory activity of the ethanolic extract of stem of *Artemisia pallens*

Concentration (µg/mL)	Absorbance at 680 nm	% Inhibition
200	0.37	65
400	0.35	67
600	0.30	72
800	0.24	77
1000	0.19	82
Control	1.06	-

Conclusions

The results of this study revealed that the extract of *Artemisia pallens* have inhibitory properties. Preliminary phytochemical screening of alcoholic extract showed the presence of various phytoconstituents (i.e., phenolics, flavonoids and saponins). The presence of substantial amount of phenolics and flavonoids are responsible for their marked antioxidant activity which is clearly revealed by PM assay. The stem extract could be a source of important chemical agents to be used to treat bacterial and fungal infections caused by *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, E. coli, Trichoderma sp., Candida albicans.* The presence of bioactive compounds like phenolics and flavonoids has the ability to inhibit pain perception and they can also serve as anti-inflammatory agents. Flavanoids act as anti-inflammatory response in the same way as non-steroidal anti-inflammatory drugs i.e., by inhibiting the enzymes that cause the synthesis of prostaglandins. This study gives an idea that the compound of the plant can be used as the lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases like cancer.

Conflicts of interest

The authors declare no conflict of interest.

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