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Full Length Research Article

Antioxidant and Antimicrobial Activity of C*rotalaria pallida* (Aiton) Seeds

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Abstract

The annual erect herb *Crotalaria pallida* Aiton, a member of the Fabaceae family is found throughout the tropics and subtropic region. *C. pallida*, also known as "rattlebox" is a plant frequently employ in conventional medicine to serve a broad range of illness, including diabetes, skin infections, snake bites, diarrhea and urinary tract illnesses. The aim of the present investigation is to evaluate the antioxidant, phytoconstituents, antibacterial, total phenol and total flavonoid effects of *C. pallida* seeds in four different solvents based on their polarities, such as aqueous, ethanol, methanol and hexane. The presence of phenols, terpenoids, saponins, alkaloids, sterols, tannins and flavonoids rich in ethanol extract. In contrast to aqueous, methanolic and hexane, the ethanol seed extract showed free radical scavenging capacity with half maximal inhibitory concentration value of 30.3µg/ml. The ethanol extract of *C. pallida* compared with standard antibiotic ciprofloxacin, *C. pallida* seeds showed that the ethanol extract had a higher total phenol content (TPC), 12.01 mg GAE/g and a greater total concentration of flavonoids (TFC), measuring 11.07 mg QE per 100 g of dry extract.

***Key words*:** *Crotalaria pallida*, Phytochemical profiling, Total phenol, Antioxidant and antimicrobial activity

The medicinal herb may provide another source for an antibiotic with considerable action against harmful germs due to the side effect of the synthetic antibiotic. *Crotalaria* from the family of Fabaceae (Leguminosae), comprises over six hundred species and is the third biggest genus in the family that naturally occur in tropical and subtropical climates across the world [1]. In India *Crotalaria* consists of 81 species of which 27 are endemic and Red Data Book of Indian Plants has a list of 15 *Crotalaria* species [2-3]. Some species of *Crotalaria* are grown ornamentally. The genus generally adapted itself to the tropical climate and only a few members are present in temperate regions [4].

Many species become harmful because pyrrolizidine alkaloids build up throughout the flowering and seed-forming stages [5]. *Crotalaria* species play a significant role in the veterinary pharmacy and also in curing many diseases [6]. *Crotalaria pallida* Aiton (Fabaceae) is commonly identified as 'rattle or rattlebox' since the jingle of its fruit when dry [7]. Here is a 1.5 m or taller erect shrub that is either a yearly or a short-lived continuing herb. The thick stem contains longitudinal grooves and is hairy. Trifoliate leaves with elliptic to obviate leaflets that are 3-13 × 2-5 cm and a 2-8.5 cm long petiole. Yellow racemes with reddish-brown veins that are 15–40 cm long and contain 20–30 blooms apiece. Fruits are lustrous, speckled ochre, 3-5 × 0.6-0.8 cm, 30-40 heart-shaped seeds, 3 × 2 mm, and dark grey-green or brown color. The plant is raised as a ground cover and a crop for green manure especially in the areas between rows of coconut palms and rubber plants.

In conventional medication, this plant is employed as a remedy for fever and urinary problems, a plaster of the roots is applied to inflammation of joints and worms in the intestine are removed using a leaf extract [8]. The people of Vanavasi hill of Salem district, Tamil Nadu, use all the plant parts to treat fever and prevent skin infections, eczema and urinary problems [9]. *C. pallida* is used to cure snake bites, diabetics, stomach aches and skin infections [10-11]. Leaf and bark paste of *C. Pallida* is used externally to treat skin diseases by the Kanitribals of Tamil Nadu [12]. Indian subcontinent's southwest coast, the leaf juice of *C. pallida* is used for the treatment of mumps [13]. Human diseases are becoming increasingly resistant to frequently used antibiotics, necessitating the hunt for novel antimicrobial compounds from other sources. The rise in drug-resistant bacterium cases and some strains decreased susceptibility to

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antibiotics made some bacterial diseases incurable and prompted researchers to look for new antibacterial chemicals in other plants [14]. Finding possible novel compounds for therapeutic purposes requires screening medicinal plants for antibacterial properties and phytochemicals, Alkaloids, phenolic compounds, and other secondary metabolites found in plants have several antibacterial activities. The present study is to evaluate the phytochemical constituents, total phenolic, antibacterial activity, total flavonoid content and antioxidant activity.

MATERIALS AND METHODS

Collection of Crotalaria pallida seed

C. pallida seed was collected from Bathalapalli village in the Koundinya Forest of Vellore district, Tamil Nadu. The plant was recognized and authenticated as *Crotalaria pallida* Aiton (Fabaceae) by Dr. S. Soosairaj, Associate Professor, Department of Botany, St. Joseph's College (Autonomous), Tiruchirapalli - 620002, Tamil Nadu. (Accession number 3053, dated 08.02.2022). Large quantities of seeds were collected, then washed under running water to remove any dirt that had become trapped. Then the seeds were dried in the shade. The dry seeds were placed in an airtight container after being mechanically mashed into a coarse powder.

Preparation of C. pallida seed extract

C. pallida seed powder weighing 40 g was dissolved in 400 ml of different solvents such as aqueous, ethanol, methanol and hexane based on their polarity and held inside a shaker orbit for 72 hours. Filtrate Whatman No. 1 was used to filter the extract to produce a particle-free extract. Using a rotary evaporator to dry after extraction at low pressure. For further investigation, the resulting ethanol extract was kept at 4 °C [15].

Phytochemical analysis

Phytochemical profiling was done according to the method of Obdoni and Ochuko [16] and Kaur and Arora [17].

DPPH radical scavenging activity

By evaluating the in vitro produced extracts DPPH free radical scavenging capacity, as expressed in Hossain *et al.* [18]. A DPPH solution was made by dissolving 4 mg of DPPH in 100 mL of methanol. 50 µl of the sample was mixed with an aliquot of 1 ml of this solution at various concentrations (12.5 g/ml to 100 g/ml). The absorbance was determined to be 517 nm after this combination had been thoroughly mixed and left in the dark for 20 minutes at room temperature. To determine the percentage of inhibition, [(Ao - A1) / Ao] × 100 was employed where A_1 is the absorbance of the extract and Ao is the absorbance of the DPPH solution used as a control. The standard is utilized as ascorbic acid (AA). After the absorption curves were produced, IC_{50} values were calculated.

Determination of antibacterial activity

The agar well diffusion technique was to ascertain the antibacterial properties of *C. pallida* seeds were examined with sterile cotton swabs and a variety of microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* and *Escherichia coli* were swabbed on nutrient agar (NA) dishes. To assess the impact in a dose-dependent way, different amounts of different extracts of *C. pallida* Aiton seed (50-200 mg/ml) were tested and the extract of different solvents was also compared with commercially available broad-spectrum antibiotics (ciprofloxacin). Ethanol, methanol, aqueous and hexane were used as a negative control. For 24 hours, the plates were incubated at 37 °C. After the incubation zone size was measured by using an antibiotic zone scale.

Determination of total phenolic content

Comparing the total phenolic content of various seed extracts was found using the Folin-Ciocalteu technique represented in Adedapo *et al*. [19]. A standard solution of gallic acid (50, 100, 150, 200, and 250 µg/ml) was used for all assays and 2mg of the extract was dissolved in 20 ml of 80% ethanol. The extracted solution (300 µl in triplicate) was combined with 1.2 ml of 7.5% (w/v) sodium carbonate and 1.5 ml of 10% folin ciocalteus reagent. After 30 min of darkness, the mixture was tested for absorbance at 765 nm. The standard curve shape for gallic acid is the basis for quantification. Gallic acid equivalent (GAE), or plant extract milligram gallic acid/gram was used to indicate the results.

Determination of total flavonoid content

The amount of total flavonoid content was determined using an aluminium chloride colorimetric assay. Briefly, six test tubes were produced with standard quercetin solutions at doses of 100, 200, 300, 400, and 500 g/ml. From each test tube, 1 ml of the solution was transferred into six other test tubes. A different test tube was filled with 0.3 ml of the sample. Following that, each test tube receives 0.3 ml of 5% $NaNO_2$ and 4 ml of distilled water. Each test tube received 0.3 ml of 10% $AlCl_3$ after 5 minutes. Each test tube was filled with 2 ml of 1M NaOH. The absorbance was measured at 510 nm after 30 minutes [20].

RESULTS AND DISCUSSION

Phytochemical analysis

This investigation's inferences sustained the usage of *C. pallida* as a traditional remedy. The data shows that seed extracts were efficient in a variety of solvents. The ethanolic extract showed a good result in phytochemicals and the aqueous and methanolic extracts similarly produced all the phytochemicals in the lesser amount shown in (Table 1, Fig 1). Tannins have a character for being effective in the treatment of diarrhea and dysentery [20]. The hexane extracts similarly produced poor and absent phytochemicals (Table 1).

Table 1 Phytochemical analysis for the different solvent extracts of *C. pallida* seeds

Constituents	Ethanol extract	Aqueous extract	Methanol extract	Hexane extract
Alkaloids	+	+	+	+
Carbohydrate	+	+	+	-
Phenol	+	+	+	-
Flavonoid	+	+	+	-
Tannins	+	+	+	-
Saponin	+	+	+	+
Terpenoid	+	+	+	-
Steroid	+	+	+	+
Glycoside	+	+	-	-

+ Present - Absent

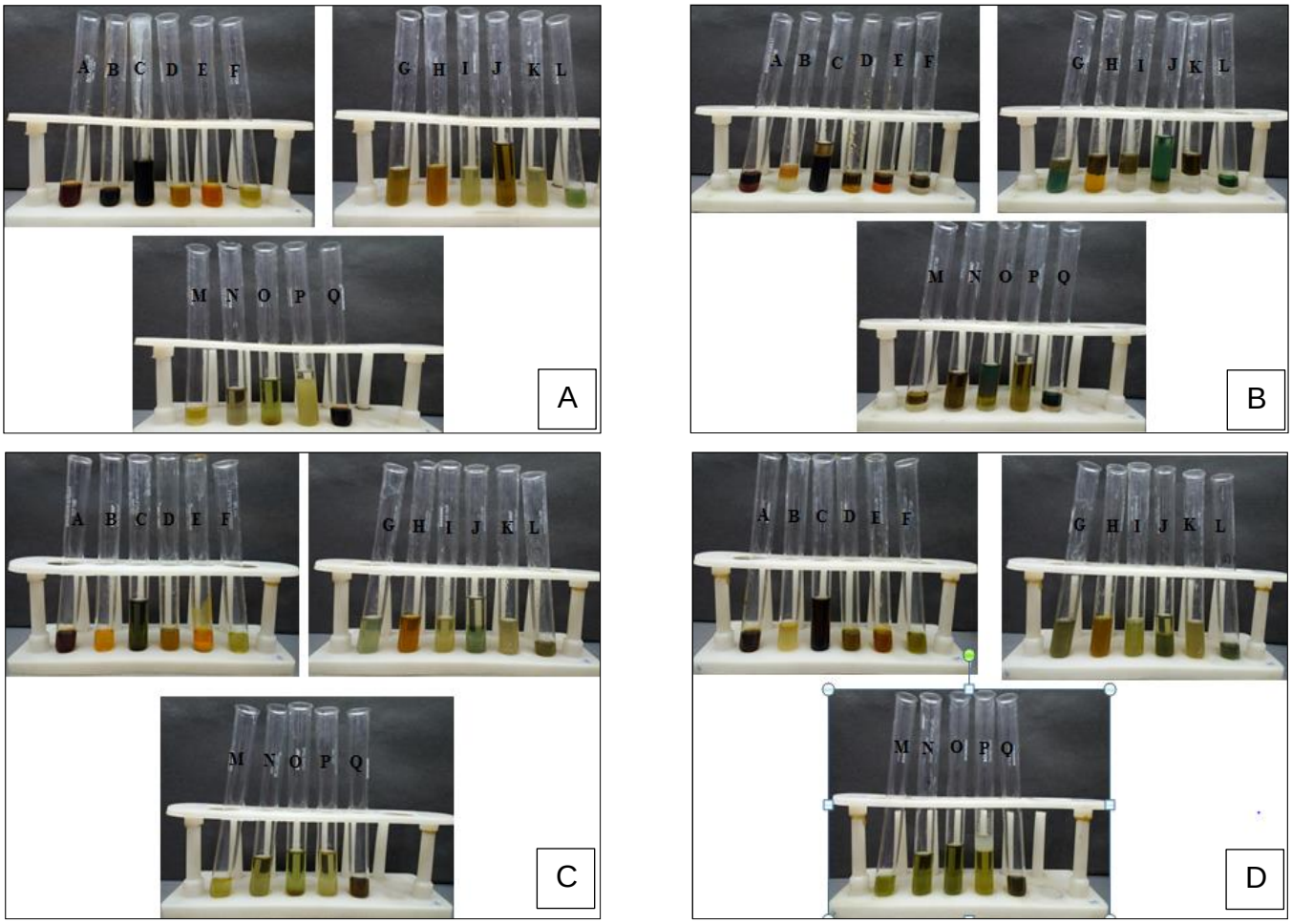


Fig 1 Phytochemical analysis of different solvent extracts of *Crotalaria pallida*
(A) Ethanolic extract (B) Aqueous extract (C) Methanol extract and (D) Hexane extract

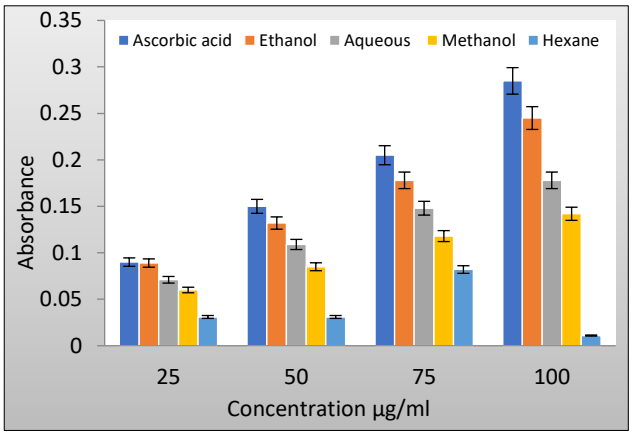
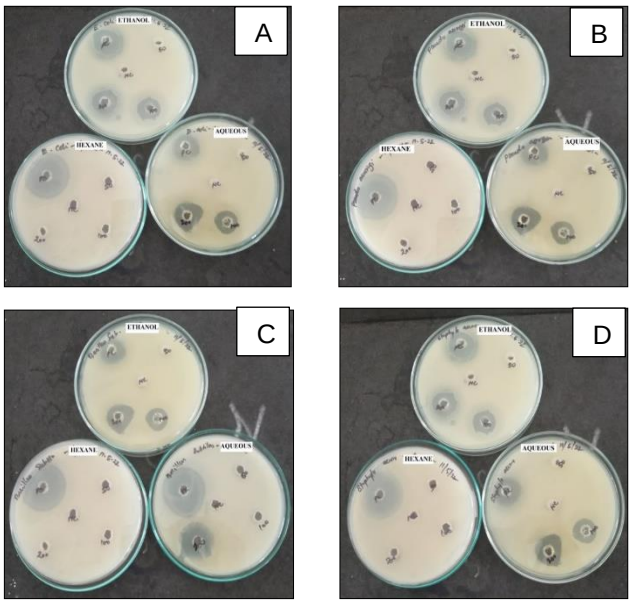


Fig 2 DPPH radical scavenging activity of different extracts of *C. pallida* seed



(A) *E. coli* (B) *P. aeruginosa* (C) *B. subtilis* and (D) *S. aureus*

Fig 3 Antibacterial activity of various extracts of *C. pallida* seeds with the zones of inhibition

DPPH radical scavenging activity

The outcome of the DPPH technique is used to determine the ability of several *C. pallida* seed extracts to evaluate scavenge free radicals, and these findings are depicted in (Fig 2). In comparison to ethanol extracts and standard ascorbic acid, the fraction of hexane extracts for inhibition increased significantly at a slower pace. The various polyphenolic constituents, such as tocopherols, flavonoids, and other organic acids, may be the responsible for the observed antioxidative effects. Recent studies have reported that polyphenols have a crucial role in antioxidant activity. The percentage of inhibition of ethanol extract increased quickly and was nearly comparable to the standard value ($y = 2.2692x + 31.135$ $R^2 = 0.9917$) and the half-maximal inhibitory concentration value of 30.34µg/ml.

Antibacterial activity

The antimicrobial properties of the *Crotalaria pallida* ethanolic seed extract was dose-dependent, peaking at a concentration of 200 mg/ml and less effect is observed at a dosage of 50 mg/ml in (Fig 3). The four bacteria which we used for antibacterial activity are resistant to commercially used antibiotics. The maximum antibacterial activity of the *C.*

pallida seed ethanol extract was found in *Escherichia coli* (16 ± 1), *Pseudomonas aeruginosa* (12.66 ± 1.52), *Bacillus subtilis* (10.76 ± 0.57) *Staphylococcus aureus* (5 ± 0.57) 200mg/ml concentration. Other extracts such as Aqueous, Methanol and Hexane extracts show decreased activity compared to ethanol extract given in (Table 2).

Table 2 Antibacterial activity of *Crotalaria pallida* seed extracts

Microorganism	Solvents	The inhibition zone in mm				
		PC	NC	50µg/ml	100 µg/ml	200 µg/ml
Escherichia coli	Ethanol	30.33 ± 0.57	NA	3.66 ± 1.52	11.33 ± 0.57	16 ± 1
	Aqueous	29.66 ± 1.52	NA	2.33 ± 0.57	7.66 ± 1.52	12.66 ± 1.52
	Hexane	30.33 ± 0.57	NA	0.83 ± 0.28	2 ± 1	3.33 ± 1.15
Pseudomonas aeruginosa	Ethanol	30 ± 1	NA	1.66 ± 0.57	8.33 ± 0.57	12 ± 1
	Aqueous	29.66 ± 1.52	NA	0.83 ± 0.28	5.66 ± 0.567	9 ± 1
	Hexane	30.33 ± 0.57	NA	NA	1.16 ± 0.76	1.66 ± 0.57
Bacillus subtilis	Ethanol	30.33 ± 0.57	NA	1.66 ± 0.57	5.66 ± 0.57	10.76 ± 0.57
	Aqueous	30.33 ± 0.57	NA	1.66 ± 0.57	3.66 ± 0.57	7.16 ± 0.76
	Hexane	29.66 ± 1.15	NA	NA	0.33 ± 0.57	0.33 ± 0.57
Staphylococcus aureus	Ethanol	29.33 ± 0.57	NA	NA	3.33 ± 0.57	5 ± 0.57
	Aqueous	30.33 ± 0.57	NA	NA	0.33 ± 0.57	1.66 ± 0.57
	Hexane	30.33 ± 0.57	NA	NA	NA	0.33 ± 0.57

n=3; mean ± SD, PC- Positive control, NC – Negative control, NA- No activity

Total phenolic content

Strong antioxidants and antimicrobials were specifically found in the seed extracts of *Crotalaria pallida.* High total phenolic content (TPC) values (9.41 and 6.81 mg GAE/100 ml) in aqueous leaf and stem extracts suggest that phenolic chemicals play a role in promoting these actions. It has been discovered that plant phenolic compounds include strong antioxidants [19]. Regarding the total phenolic content (TPC), the ethanolic extract of *Crotalaria pallida* has the highest value (12.01mg GAE/g), followed by aqueous (10.8mg GAE/g), methanol (9.95mg GAE/g) and hexane (6.33mg GAE/g) shown in (Fig 4).

Determination of total flavonoids content

Recent research has revealed that several flavonoids and associated with polyphenols greatly provide the anti-inflammatory and antioxidant properties of various plants. The presence of biologically active constituents in the ethanol extract of *Crotalaria pallida* sections may be responsible for the plant's antibacterial, anti-inflammatory and antioxidant properties. The results were derived from the calibration curve ($y = 0.0026x + 0.0497$, $R^2 = 0.9923$) of quercetin shown in (Fig 5). The flavonoid content in ethanol extracts is 11.07 mg QE/g, aqueous 10.09 mg QE/g, methanol 8.97 mg QE/g, and in hexane 3.2 mg QE/g respectively.

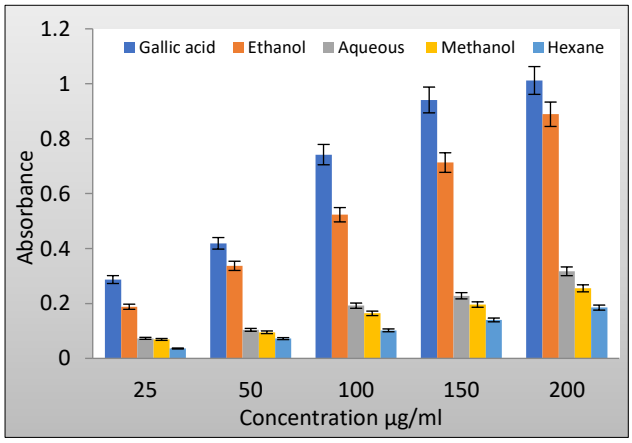


Fig 4 Determination of total phenolic content of *Crotalaria pallida* seed extracts

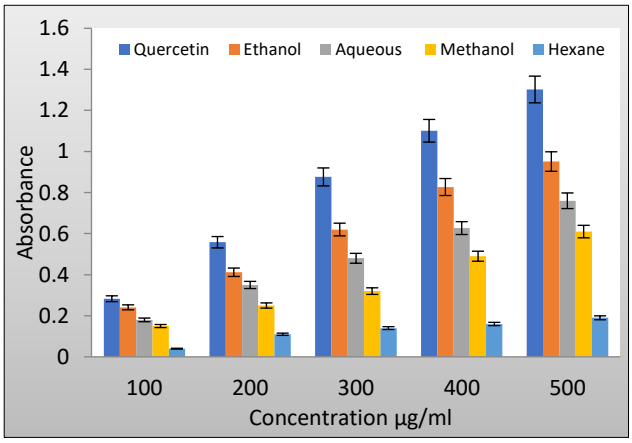


Fig 5 Determination of total flavonoid content of *Crotalaria pallida* seed extract

CONCLUSION

The current study analysis shows that different solvent extracts of *Crotalaria pallida* seeds exhibit antioxidant and antibacterial properties as well as several primary and secondary metabolites, including tannins, saponins, terpenoids, phenols, and flavonoids. *Crotalaria pallida* extract has a diverse array of Gram-negative bacteria activities *Escherichia coli*, *Pseudomonas aeruginosa*, and Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* which cause the most common bacterial diseases. Comparing the ethanolic extract with the reference antibiotic ciprofloxacin showed more significant activity in *Crotalaria pallida* seeds ethanolic extract (12.01 mg GAE/g) indicates a particular connection between the number of phenols in a sample and the IC_{50} value for antioxidant activity Half-maximal inhibitory concentration value is 30.34µg/ml. So, this plant will help in the development of antibacterial and antioxidant compounds.

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