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Research Article

## PRELIMINARY PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL STUDIES ON LEAF EXTRACT OF *CROTALARIA HEBECARPA*

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Bacterial illnesses have presented significant threats to both animal husbandry and human health globally. The leaf of *Crotalaria Hebecarpa* from the family Fabaceae were studied to give a final report on preliminary phytochemical screening and in-vitro anti-bacterial and anti-fungal activity. The plant's leaves were air dried, ground into powder, and then extracted with 70% methanol. The resulting product was then filtered and dried at 50 degrees Celsius to form a dark green semi-solid mass. The methanolic extract of *Crotalaria hebecarpa* exhibited antibacterial activity against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, as well as two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. A quantitative micro spectrophotometric test was used to determine antifungal activity. The suppression of growth was quantified in 96-well microtiter plates at a wavelength of 595 nm. Tests were regularly conducted using 20 µL of the extract for analysis, 10 µL of a spore suspension, and 70 µL of potato dextrose broth (PDB) (HiMedia, Mumbai, India). Using a microplate reader at 595 nm, the MIC values were done spectrophotometrically. The MIC of the extracts was determined as the minimal concentration of plant extract that resulted in over 90% growth inhibition at 48 hours, relative to the control. In this study, *Crotalaria hebecarpa* was evaluated for its antibacterial and antifungal efficacy against *Klebsiella*, *E. coli*, *Proteus* spp., *Streptococcus*, *Staphylococcus aureus*, and *Bacillus* spp. None of the previous investigations have strictly tested the potential of the Methanolic leaf extract. The leaf extract of *Crotalaria hebecarpa* demonstrates significant potency, exhibiting dose-dependent antibacterial and antifungal activities. The methanolic extract of *Crotalaria hebecarpa* at a concentration of 10 µg demonstrates optimal efficacy against all pyrogens, particularly *Streptococcus pyogenes* (excluding *Bacillus* species). *Crotalaria Hebecarpa* has been validated to exhibit considerable antibacterial and antifungal properties, with the methanolic extract demonstrating encouraging efficacy. Further research may concentrate on extracting and identifying the active elements responsible for its pharmacological actions, thereby facilitating the development of herbal-based antibacterial and antifungal agents.

**Keywords:** *Crotalaria hebecarpa*, Anti-bacterial, Anti-fungal Anti-oxidant.**Corresponding author:****K.L.Senthil Kumar ,**

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## 1. INTRODUCTION:

All over the world, bacterial infections have been one of the greatest dangers to human health and animal husbandry [1]. There are approximately twelve classes of recognized pathogenic bacteria that cause an array of diseases. Because these bacteria are becoming more resistant to different antimicrobial medications, there is a need to screen for new lead molecules to treat these bacterial pathogens. One Gram-negative, obligatory aerobic bacteria that can cause nosocomial infections is *Acinetobacter baumannii*, also known as coccobacillus. It exhibited resistance to multiple major antimicrobial drugs and has contributed to the lethal confluence of pathogenicity and antibiotic resistance that afflicts hospitals. A Gram-positive bacteria, *Listeria monocytogenes* is an intracellular pathogen that is nearly universal and has been linked to a number of disease outbreaks in the past [2]. The herb possesses anti-inflammatory, antioxidant, and antibacterial qualities. To the best of our knowledge, *Crotalaria hebecarpa* leaves have not yet been the subject of many pharmacological investigations [3].

## 2. METHODS

### 2.1 SAMPLE PREPARATION

The plant *Crotalaria Hebecarpa*, a member of the "Fabaceae" family, is widely available in Tamil Nadu. For the current investigation, the plant *Crotalaria Hebecarpa* was collected from the vicinity of Dharmapuri. The plant was recognized and its authenticity confirmed by an expert. The leaves were cut with a knife and then left in the shade for two weeks to air dry. The dried leaves were ground into a mesh and kept in an airtight container for subsequent use.

### 2.2 PROXIMATE ANALYSIS

The moisture content, total ash content, water soluble ash content, and acid insoluble ash content of the generated sample were evaluated by proximate analysis using the previously mentioned standard procedures [4]. Extract preparation: The extracts of *Crotalaria Hebecarpa* leaves were made using the sequential extraction method. This method involved extracting the sample materials one at a time using a number of solvents in ascending order of polarity (from non-polar to polar). The prepared sample (120 grams) was extracted progressively in different solvents, including petroleum ether, chloroform, methanol, and distilled water, for 10 hours using a Soxhlet apparatus [5]. These extracts were concentrated using a rotary evaporator, dried in an oven dryer set at 35 to 40 °C, and then stored for further use.

### 2.3 PHYTOCHEMICAL SCREENING

A preliminary phytochemical screening of the various plant extracts was carried out in line with the

conventional procedures that had been published in the past. The purpose of this screening was to determine whether or not the extracts included active chemical components such as alkaloids, flavonoids, saponins, tannins, proteins and amino acids, and phenolic compounds. [6]

## 2.4 ANTIOXIDANT ACTIVITY

The measurement of antioxidant activity is inherently complicated due to the intricacies of biological systems. The DPPH scavenging activity, reducing power assay, total antioxidant capacity, and ferric ion reduction were employed to evaluate the antioxidant properties of the various extracts under examination.

### 2.5 DPPH SCAVENGING ACTIVITY

The potential of the obtained extracts to scavenge free radicals was assessed in vitro using DPPH free radicals. 100 milliliters of methanol were utilized to dissolve 4 milligrams of DPPH to prepare the DPPH solution. 50 µl of the sample at several concentrations (31.25 to 500 µg/ml) was mixed with an aliquot of 1 ml of this solution. Following a thorough agitation of this mixture and a 20-minute incubation at ambient temperature in the absence of light, the absorbance at 517 nm was recorded. The absorbance of the control (DPPH solution) is denoted as  $A_0$ , whereas  $A_1$  represents the absorbance of the extract/standard. The percentage inhibition was calculated using the formula  $[(A_0 - A_1)/A_0] \times 100$ . BHT and ascorbic acid (AA) were utilized as standards. The formulation of inhibition curves and the calculation of IC<sub>50</sub> values.

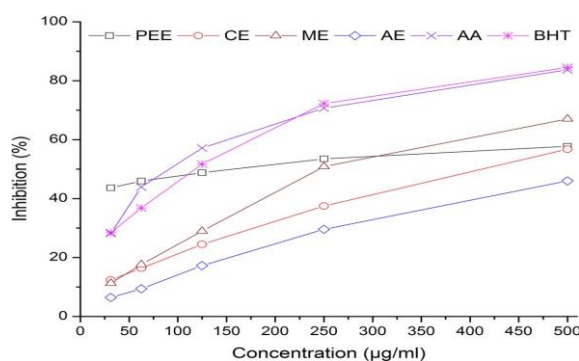


Fig.1 DPPH radical scavenging activity of different extracts of *Crotalaria Hebecarpa* and standard antioxidants.

### 2.6 REDUCING POWER ASSAY

The capacity of the produced extracts to reduce ferric ions to ferrous ions was assessed by quantifying the formation of Perl's Prussian blue at 700 nm, following the previously outlined procedure. This facilitated the assessment of the reducing power of the extracts. A 1% potassium ferricyanide solution was combined with 1 ml of

phosphate buffer (0.2 M, pH 6.6) and extracts at concentrations from 31.25 to 500 µg/ml. Following a 20-minute incubation at 50 °C, 2 milliliters of 10% trichloroacetic acid were introduced to the mixture. After centrifugation of the mixture for 10 minutes at 3000 rpm, a volume of 0.5 ml of the supernatant from each sample was combined with 1 ml of distilled water and 0.1 ml of 0.1% (w/v) ferric chloride. A spectrophotometer measured the absorbance at 700 nm relative to a blank after a 10-minute reaction period. The reaction mixture exhibited enhanced reducing power, as indicated by increased absorbance. The employed reference standard was BHT.

## 2.7 TOTAL ANTIOXIDANT CAPACITY

The phosphomolybdenum technique, with minor adjustments, was employed to spectrophotometrically evaluate the total antioxidant capacities of all samples. One millilitre of reagent solution, comprising 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, was combined with 100 µl of sample solution in an Eppendorf tube. The sealed tubes underwent incubation for 90 minutes at a temperature of 50 °C. Absorbance at 695 nm was recorded against a blank after the samples equilibrated to room temperature. A standard blank solution was prepared by incorporating one millilitre of reagent solution and the appropriate quantity of solvent used for the sample, followed by incubation under identical conditions as the other samples. The overall antioxidant activity was expressed in ascorbic acid equivalents, using AA as the reference standard.

## 2.8 REDUCTION OF FERRIC IONS ASSAY

The capacity of the extracts to reduce ferric ions was evaluated using the established o-phenanthroline method. Following a 10-minute incubation at room temperature, a reaction mixture consisting of 1 millilitre of o-phenanthroline (5 mg in 10 millilitres of methanol), 2 millilitres of 0.2 mM ferric chloride, and 2 millilitres of extracts at varying concentrations (31.25 to 500 µg/ml) was evaluated for absorbance at 510 nm. AA and gallic acid (GA) were utilized as reference standards.

## 3. RESULTS:

### 3.1 ANTIOXIDANT ACTIVITY

Substances that considerably slow down or prevent the oxidation of simple oxidisable substrates, even at low concentrations, are known as antioxidants. It functions by either lowering or chelating the transition metal content or by stopping the body from producing free radicals.

#### 3.1.1 DPPH RADICAL SCAVENGING ACTIVITY

The DPPH method was used to see if the extracts of *Crotalaria Hebecarpa* leaves could get rid of free radicals. The results are given in Fig. 1. Even though the rate was much lower than that of other extracts and standards, the percentage of inhibition by PEE went up in a straight line as the concentration went up. On the other hand, isolated extracts slowly increased the amount of inhibition, which was almost the same as the standards. The data, which are provided, clearly reveal that the AE was very high (898.46 µg/ml) and that the 50% scavenging activity (IC<sub>50</sub>) of PEE was substantially lower (126.96 µg/ml) than that of other extracts. A lower IC<sub>50</sub> value means that the antioxidant activity is higher. The purpose of antioxidants is to interact with free radicals that cause oxidation. The basic premise behind the DPPH method is that antioxidants react with the stable free radical 2,2-diphenyl-1-picrylhydrazyl, which is a deep violet color, to make 2,2-diphenyl-1-picrylhydrazine, which is a colorless compound. The amount of discoloration shows how well the antioxidant sample can scavenge. The current study found that *Crotalaria Hebecarpa* leaf extracts could remove color from DPPH. The order of their abilities to scavenge free radicals was PEE > ME > CE > AE. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.), and aromatic amines (p-phenylene diamine, p-aminophenol, etc.) all lower and decolorize DPPH by giving it hydrogen. It appears that extracts from the leaves of *Crotalaria Hebecarpa* work as antioxidants via utilizing hydrogen-donating characteristics.

Table No-1: Inhibition of concentration

| S.no | Extract | Concentration |
|------|---------|---------------|
| 1    | PEE     | 127.11        |
| 2    | CE      | 455.6         |
| 3    | ME      | 255.13        |
| 4    | AE      | 898.46        |
| 5    | AA      | 90.06         |
| 6    | BHT     | 100.05        |

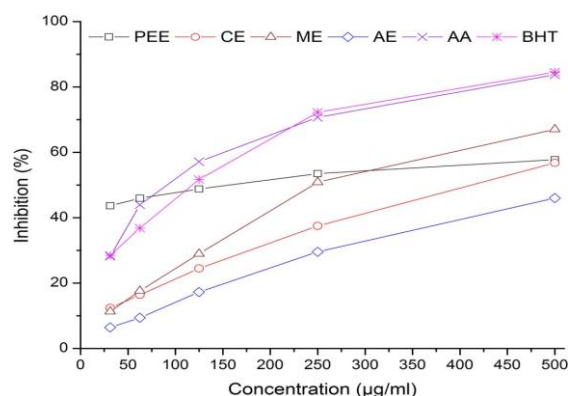


Fig.2 DPPH radical scavenging activity

### 3.2 ANTIBACTERIAL ACTIVITY

The methanolic extract of *Crotalaria hebecarpa* exhibited antibacterial activity against two Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. Gentamycin was used as one of the positive controls. It shows the results of the antimicrobial test. *Crotalaria hebecarpa* extract stopped all of the microorganisms used in this study, except for *B. cereus*. The ranges for the zones were from 13 to 26 mm. The extract was most effective against *E. coli*, which had a 22 mm zone of inhibition. *P. aeruginosa* was the least sensitive, with a 13 mm zone of inhibition. *B. cereus* showed no sensitivity to any of the extracts that were tested. The reasons why

different solvent extracts work differently have not been looked into yet, although it could be because the active components don't all dissolve equally well in the solvents. The ineffectiveness against bacteria might stem from the solvents' antagonistic interactions with the constituents of the *Crotalaria hebecarpa* extract. Tannins and flavonoids can bind to proteins in many ways, create complexes with bacterial cells, and even inhibit enzymes, which can kill bacteria. Studies show that most hazardous bacteria, like *S. aureus* and *E. coli*, that are found on the hands of healthcare workers are resistant to many different types of antimicrobial treatments. However, this plant extract has been shown to work moderately to strongly against various antimicrobial medicines.

**Table No-2 Zone of inhibition of extract**

| S.No | Bacterial Strain      | Zone of inhibition |    |    |
|------|-----------------------|--------------------|----|----|
|      |                       | A                  | B  | C  |
| 1    | <i>Klebsiella sp.</i> | 22                 | 26 | 18 |
| 2    | <i>E.coli</i>         | 21                 | 22 | 19 |
| 3    | <i>Proteus sp.</i>    | 19                 | 21 | 19 |
| 4    | <i>S.aureus</i>       | 20                 | 23 | 24 |
| 5    | <i>Streptococcus</i>  | 15                 | 18 | 26 |
| 6    | <i>Bacillus Sp</i>    | 13                 | 18 | 28 |

A-30ul, B-40ul, C-Gentamycin

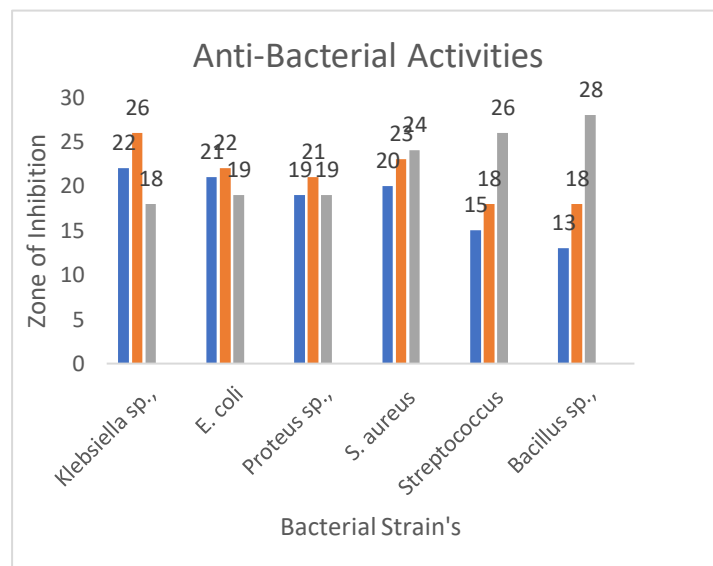


**Fig.3 Antibacterial activities**

### 3.3 ANTIFUNGAL ACTIVITY

A quantitative micro spectrophotometric assay was employed to evaluate antifungal efficacy. Growth inhibition was measured at 595 nm in 96-well microtiter plates. Tests were done as usual with 70  $\mu$ L of potato dextrose broth (PDB) (HiMedia, Mumbai, India), 10  $\mu$ L of a spore suspension, and 20

$\mu$ L of the extract that was going to be tested. Microcultures containing 20  $\mu$ L of sterile distilled water were utilized as a negative control in lieu of the test solution. We used 0.2 mg mL<sup>-1</sup> of the commercial fungicide captan (N-(trichloromethylthio) cyclohex-4-ene-1,2-dicarboximide) as a positive control. After letting

the spores settle for 30 minutes at 27 °C, the plates were put in an ELISA plate reader to check the absorbance at 595 nm. During a 48-hour incubation period at 27 °C, absorbance was used to quantify growth. There were at least three tests for each antifungal activity. We used the calculation  $[(\Delta C - \Delta T)/\Delta C] \times 100$  to figure out how much the growth was stopped.  $\Delta C$  is the corrected absorbance of the control microculture at 595 nm, and  $\Delta T$  is the corrected absorbance of the test microculture. To get the corrected absorbance values, you take the absorbance at 595 nm after 48 hours and remove the absorbance at 595 nm after 30 minutes. The minimal fungicidal concentration (MFC) and the minimum inhibitory concentration (MIC) The microplate method described before was used with a few small changes to find the plant extracts' minimum inhibitory concentrations (MICs). The crude extract was diluted in serial steps from 1/2 to 1/100. Each well had 100  $\mu$ L of each extract dilution and 100  $\mu$ L of the fungal spore suspension ( $2 \times 10^6$  spores mL<sup>-1</sup> in fresh PDB). Every day, the microplates were

checked on while they were being incubated at 27 °C for two to three days. Three times, we did each experiment. The MIC measurements were obtained at 595 nm using a microplate reader. To find the MIC values, we compared the growth in control wells with the extract blank, which was made up of plates that had not been infected. The minimum inhibitory concentration (MIC) of the extracts was the lowest amount of plant extract that, when compared to the control, stopped growth by more than 90% after 48 hours. Espinel-Ingroff documented the in vitro fungicidal activity (MFC). After 72 hours of incubation, 20  $\mu$ L was subcultured onto PDA plates from each well that showed no growth (growth inhibition of more than 98%), the last positive well (growth similar to that of the growth control well), and the growth control (extract-free medium). The plates were kept at 27 °C until the growth control subculture started to develop. The minimum fungicidal concentration was the lowest extract concentration that stopped all fungal growth on the solid medium.

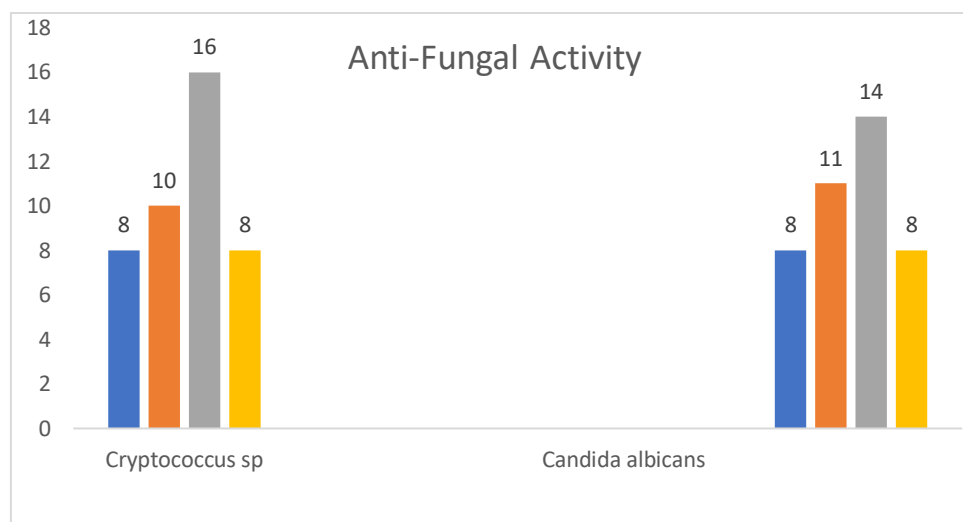


**Fig.4 Anti-Fungal activity**

### 3.4 IN VIVO BIOSAFETY ASSAY OF AMP2 AGAINST CELL LINES AND ZEBRAFISH

The safety was assessed using zebrafish as an animal model, and the AMP2-treated zebrafish challenged with *A. baumannii* had a good survival rate. It was found that 25  $\mu$ M AMP2 increased the survival rate of fish with bacterial infections, which was  $73 \pm 6.5\%$  after 72 hours and  $68 \pm 7.3\%$  after 96 hours of treatment.

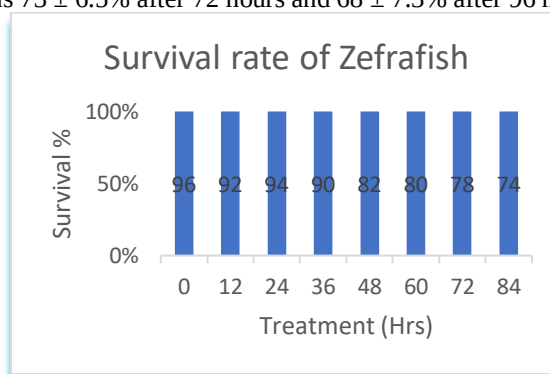


**Fig.5 Survival rate of Zebra fish challenged with *A. baumannii* and treated using 25  $\mu$ M AMP2 for 96 hrs.**  
The values are the result of three different experiments ( $\pm$  SD).

## 6.CONCLUSION:

The leaf of *Crotalaria Hebecarpa* from the Fabaceae family was examined to deliver a comprehensive report on initial phytochemical screening and in vitro antibacterial and antifungal activity. We established pharmacognostical assessments and physicochemical criteria like ash levels, extractive values, and loss on drying. This helped us properly identify and verify the plant for future use. The current findings and previous literature demonstrate that *Crotalaria Hebecarpa* has substantial antibacterial and antifungal properties, with the methanolic extract demonstrating promising action. Additional research may concentrate on extracting and identifying the active ingredients responsible for its pharmacological actions, thereby facilitating the development of herbal-based antibacterial and antifungal agents.

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