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Antibacterial Activity of Ethyl Acetate Extracts from Stem and Root of *Crotalaria ramosissima* Roxb. (Family-Fabaceae)

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Abstract
The present study aimed to evaluate the antibacterial potential in the ethyl acetate of stem and root *Crotalaria ramosissima* Roxb. against the selected pathogenic microorganisms. Plant extracts were subjected to antibacterial screening against six clinically important bacterial strains comprising three Gram-positive bacteria and three Gram-negative bacteria. The Gram-positive bacteria included *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecium*, while the Gram-negative bacteria included *Enterobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antibacterial activity was evaluated using the well diffusion method and the zone of inhibition was measured to determine the effectiveness of the extracts. The results demonstrated that the extracts of *C. ramosissima* exhibited considerable antibacterial activity against both Gram-positive and Gram-negative bacterial strains. Among the tested organisms, Gram-positive bacteria showed comparatively higher sensitivity to the plant extracts than Gram-negative bacteria, which may be attributed to differences in cell wall composition. The presence of bioactive phytochemicals such as alkaloids, flavonoids, tannins, phenolic compounds, and terpenoids could be responsible for the observed antibacterial effects. The study highlights the medicinal significance of *C. ramosissima* as a potential natural source of antimicrobial agents. These findings suggest that the plant possesses promising antibacterial properties and may be further explored for the development of novel phytotherapeutic drugs to combat microbial infections and antibiotic resistance.

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1. INTRODUCTION

The diverse medicinal plants, *Crotalaria ramosissima* has attracted scientific interest because of its rich phytochemical composition and traditional medicinal importance. *Crotalaria ramosissima* belongs to the family Fabaceae, one of the largest and economically important plant

families known for possessing several biologically active compounds. Species of the genus *Crotalaria* are widely distributed in tropical and subtropical regions and are commonly used in traditional systems of medicine for treating fever, inflammation, skin disorders, wounds, and microbial infections. Various species of *Crotalaria* have been reported to possess antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, and hepatoprotective activities due to the presence of secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, terpenoids, saponins and glycosides, (Chouhan and Singh, 2010).

The increasing prevalence of bacterial infections caused by resistant pathogens has become a serious threat to public health. Gram-positive and Gram-negative bacteria are among the major groups of pathogenic microorganisms responsible for various infectious diseases in humans. Gram-positive bacteria possess a thick peptidoglycan layer in their cell wall, whereas Gram-negative bacteria contain an additional outer membrane rich in lipopolysaccharides, making them comparatively more resistant to antimicrobial agents, (Hemendra *et al*., 2011). The structural differences between these bacterial groups influence their susceptibility to antibiotics and plant-derived compounds. Therefore, evaluating the antibacterial activity of plant extracts against both Gram-positive and Gram-negative bacteria is essential to understand their broad-spectrum therapeutic potential.

In the present study, the antibacterial activity of ethyl acetate extracts obtained from the stem and root of *Crotalaria ramosissima* was investigated against six bacterial pathogens, including three Gram-positive and three Gram-negative bacteria. The selected The Gram-positive bacteria included *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Enterococcus faecium*, while the Gram-negative bacteria included *Enterobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* These bacterial species are clinically significant pathogens associated with several human infections such as urinary tract infections, respiratory infections, wound infections, gastrointestinal disorders, septicemia and hospital-acquired infections.

Extraction is an important step in the isolation of bioactive compounds from medicinal plants. The choice of solvent greatly influences the extraction efficiency and biological activity of plant constituents. Ethyl acetate is considered an effective solvent for extracting moderately polar phytochemicals such as flavonoids, phenolic compounds, and alkaloids. Compared to aqueous extracts, ethyl acetate extracts often exhibit enhanced antimicrobial activity due to the efficient recovery of active metabolites. In this study, ethyl acetate was used for extracting bioactive compounds from the stem and root parts of *Crotalaria ramosissima* to evaluate their antibacterial potential, (Kwaji *et al*., 2013).
Several studies have reported on the antimicrobial properties of species belonging to the genus *Crotalaria*. Extracts from different *Crotalaria* species have shown inhibitory effects against bacterial and fungal pathogens due to the presence of pyrrolizidine alkaloids, flavonoids, and phenolic compounds. However, limited information is available regarding the antibacterial activity of the ethyl acetate extracts of the stem and root of *Crotalaria ramosissima*. Therefore, the present investigation was undertaken to evaluate the antibacterial efficacy of this medicinal plant against selected pathogenic bacteria and to contribute to the scientific validation of its traditional uses.

2. MATERIALS AND METHODS

2.1 Antibacterial Activity

Antibacterial activities of Sample Name were studied. Six bacterial pathogenic strains were obtained. Antibacterial property refers to the capacity to destroy the advancement of bacteria by an agent. The study of antibacterial property of a substance is crucial in controlling, fighting and eradicating the diseases caused by pathogenic bacteria in plants, animals and humans. In general, antibiotics are the well-known drugs to handle the bacteria induced pathological conditions, of course often with side effects. There are natural substances produced by plants, for instance, may secondary metabolites which exhibit antibacterial activities with less or no side effects (Dheeraj *et al*., 2024;

Naser *et al*., 2025). The bacterial strains used were *Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa, Klebsiella pneumoniae and Enterobacter freundii.* The effect of sample extracts on the six bacterial pathogen strains was assayed by disk diffusion method (Eloff, 1999).

2.2 Principle

The antibacterial agents present in the Sample extracts were allowed to diffuse out into the medium and interact in a freshly seeded plate with the test organisms. The resulting zone of inhibition will be uniformly circular as there is a confluent lawn of growth. The diameter of the zone of inhibition can be measured in millimeters.

2.3 Muller Hinton Agar Medium

The medium was prepared by dissolving 38 g of commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 90 mm Petri plates (25-30 mL/plate).

2.4 Disk Diffusion Method

Petri plates that contained 20ml of Muller Hinton agar media were inoculated with a 24-hour culture of bacterial strains. Different concentrations of 10-30 µl of sample name were applied. The plates were then placed in an incubator overnight at a temperature of 37°C for 24 hours. The diameter of the inhibition zone that formed around the well was measured to assess the antibacterial activity. These were compared with the zone of inhibitions of standards (Streptomycin 3 mg/ml) and control (DMSO).

3. RESULTS AND DISCUSSIONS

The antibacterial activity observed in this study can be correlated with the phytochemical composition of *Crotalaria ramosissima*. Comparison between stem (Table: 1 and 2) and root (Table: 3 and 4) extracts revealed that both stem and root extract consistently produced larger inhibition zones against most bacterial strains. This observation suggests that the *Crotalaria ramosissima* contains higher concentrations of active phytoconstituents in both root and stems. Ethyl acetate, being a moderate polar solvent, is effective in extracting compounds such as flavonoids, alkaloids, phenolics, and terpenoids that possess strong antimicrobial properties. Therefore, the enhanced activity of the stem (Figure: 1 and 2) and root (Figure: 3 and 4) extract may be due to the efficient extraction of these bioactive compounds. The combined action of the phytochemicals may have contributed to the broad-spectrum antibacterial activity observed against both Gram-positive and Gram-negative bacteria.

The results of the present investigation are consistent with earlier reports on the antimicrobial properties of medicinal plants belonging to the Fabaceae family. Several species of the genus *Crotalaria* have been reported to possess antibacterial activity against pathogenic microorganisms due to the presence of bioactive alkaloids and flavonoids. Previous studies have demonstrated that extracts of *Crotalaria* species effectively inhibited bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The present findings further support the therapeutic importance of this genus and provide scientific evidence for the antibacterial potential of *Crotalaria ramosissima*.

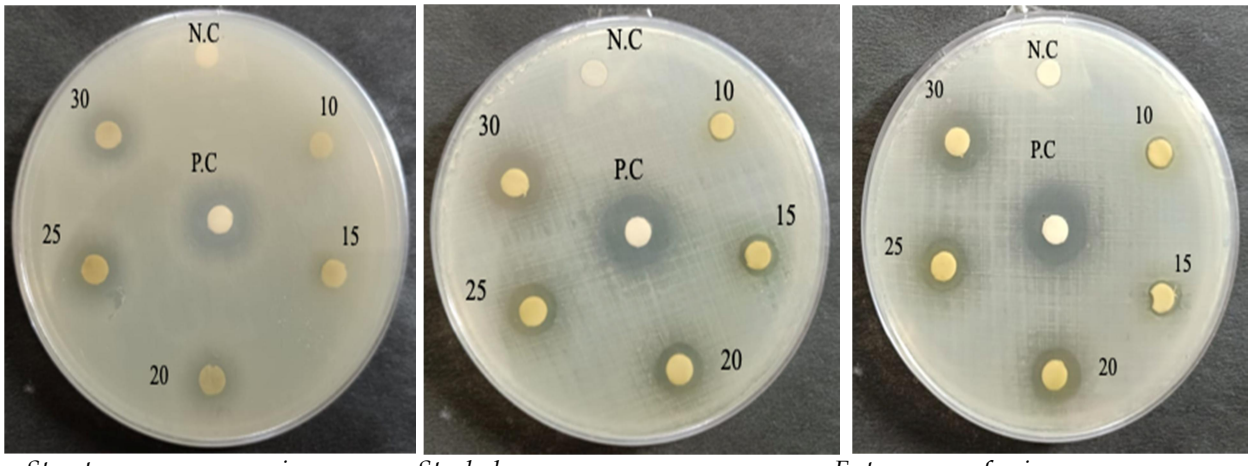
The growing problem of antibiotic resistance has increased the demand for alternative antimicrobial agents from natural sources. Synthetic antibiotics often cause adverse side effects and may lead to the development of resistant microbial strains, (Sushobhan *et al*., 2015). Plant-derived antimicrobial compounds offer several advantages, including lower toxicity, biodegradability, cost-effectiveness and reduced chances of resistance development. The antibacterial activity demonstrated by the ethyl acetate extracts of *Crotalaria ramosissima* suggests that the plant could serve as a promising candidate for the development of herbal antimicrobial formulations.

In discussion, the ethyl acetate extracts of stem and root of *Crotalaria ramosissima* exhibited significant antibacterial activity against three Gram-positive bacteria (*Staphylococcus aureus*,

Streptococcus pneumoniae, and *Enterococcus faecium*) and three Gram-negative bacteria (*Enterobacter freundii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*), (Hala *et al*., 2024). The root extract demonstrated comparatively stronger antibacterial effects than the stem extract, suggesting a higher concentration of bioactive compounds in the roots. Gram-positive bacteria were generally more susceptible than Gram-negative bacteria due to differences in cell wall structure. The observed antibacterial activity may be attributed to the presence of phytochemicals such as flavonoids, alkaloids, tannins, phenolics, and terpenoids. These findings support the medicinal importance of *Crotalaria ramosissima* and highlight its potential as a natural source of antimicrobial agents for future pharmaceutical applications, (Mathews *et al*., 2015).

Table 1: Gram - positive bacteria in the Ethyl acetate analysis of Stem - *C. ramosissima*

Ethyl acetate of Stem			
Concentration (10mg/ml)	**Zone of inhibition *(Streptococcus pneumoniae)***	**Zone of inhibition *(Staphylococcus aureus)***	**Zone of inhibition *(Enterococcus faecium)***
Negative control (DMSO)	-	-	-
10 (100 µg)	-	-	-
15 µl (150 µg)	-	6 mm	-
20 µL (200 µg)	5 mm	8 mm	9 mm
25 µL (250 µg)	8 mm	10 mm	10 mm
30 µL (300 µg)	10 mm	10 mm	12 mm
Positive control 30 µL (90 µg) (Streptomycin 3mg/ml	12 mm	14 mm	15 mm

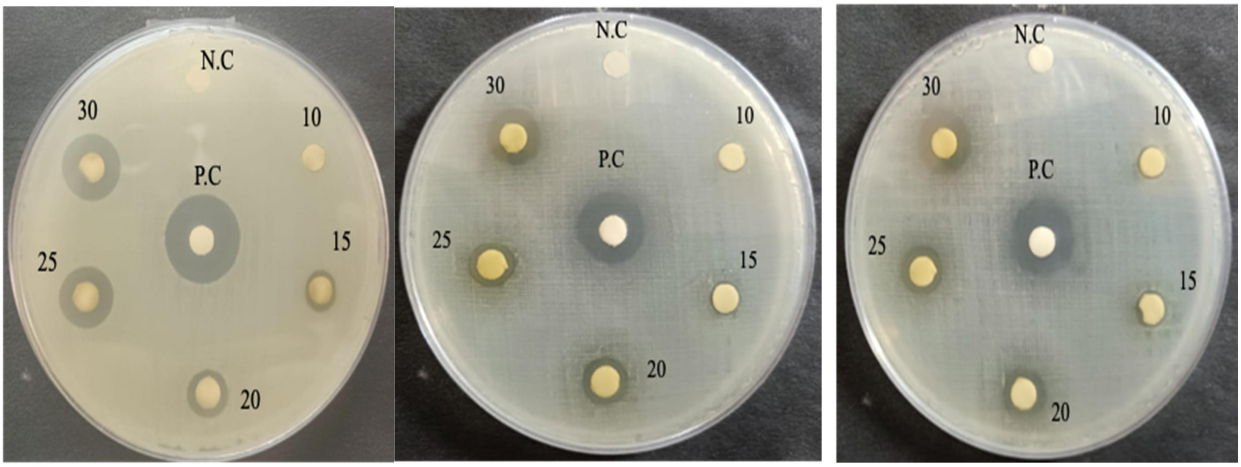


Streptococcus pneumoniae *Staphylococcus aureus* *Enterococcus faecium*

Figure 1: Gram - positive bacteria in the Ethyl acetate analysis of Stem - *C. ramosissima*

Table 2: Gram - negative bacteria in the Ethyl acetate analysis of Stem - *C. ramosissima*

Concentration (10mg/ml)	Ethyl acetate of Stem		
	Zone of inhibition *(Pseudomonas aeruginosa)*	**Zone of inhibition *(Klebsiella pneumoniae)***	**Zone of inhibition *(Enterobacter freundii)***
Negative control (DMSO)	-	-	-
10 (100 µg)	-	-	-
15 µl (150 µg)	4 mm	4 mm	5 mm
20 µL (200 µg)	8 mm	8 mm	7 mm
25 µL (250 µg)	10 mm	10 mm	8 mm
30 µL (300 µg)	13 mm	11 mm	11 mm
Positive control 30 µL (90 µg) (Streptomycin 3mg/ml	16 mm	13 mm	14 mm

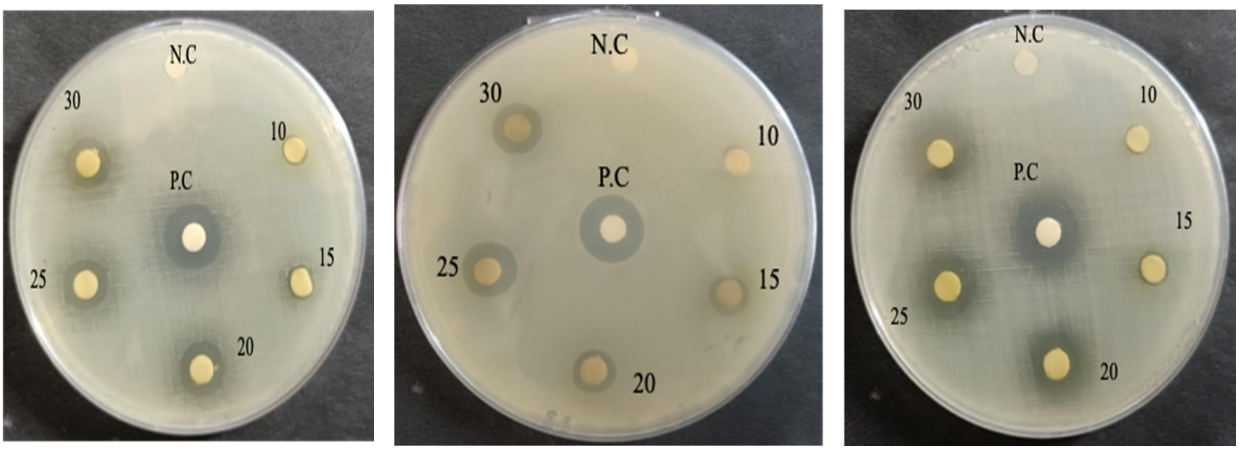


Pseudomonas aeruginosa *Klebsiella pneumoniae* *Enterobacter freundii*

Figure 2: Gram - negative bacteria in the Ethyl acetate analysis of Stem - *C. ramosissima*

Table 3: Gram - positive bacteria in the Ethyl acetate analysis of Root - *C. ramosissima*

Ethyl acetate of Root			
Concentration (10mg/ml)	**Zone of inhibition *(Streptococcus pneumoniae)***	**Zone of inhibition *(Staphylococcus aureus)***	**Zone of inhibition *(Enterococcus faecium)***
Negative control (DMSO)	-	-	-
10 (100 µg)	-	-	-
15 µl (150 µg)	5 mm	6 mm	4 mm
20 µL (200 µg)	9 mm	8 mm	9 mm
25 µL (250 µg)	10 mm	10 mm	11 mm
30 µL (300 µg)	11 mm	11 mm	13 mm
Positive control 30 µL(90 µg) (Streptomycin 3mg/ml	12 mm	14 mm	15 mm

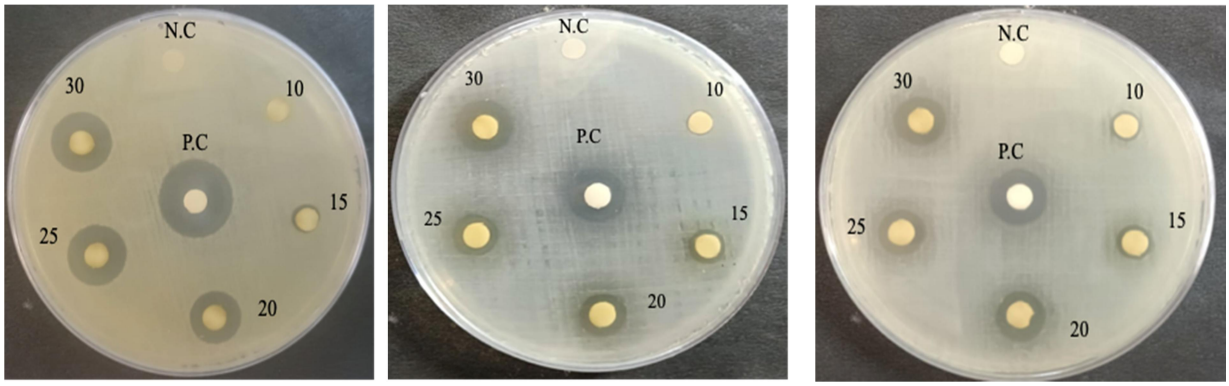


Streptococcus pneumoniae *Staphylococcus aureus* *Enterococcus faecium*

Figure 3: Gram - positive bacteria in the Ethyl acetate analysis of Root - *C. ramosissima*

Table 4: Gram - negative bacteria in the Ethyl acetate analysis of Root - *C. ramosissima*

Concentration (10mg/ml)	Root		
	Zone of inhibition *(Pseudomonas aeruginosa)*	**Zone of inhibition *(Klebsiella pneumoniae)***	**Zone of inhibition *(Enterobacter freundii)***
Negative control (DMSO)	-	-	-
10 (100 µg)	-	-	5 mm
15 µl (150 µg)	5 mm	5 mm	6 mm
20 µL (200 µg)	11 mm	8 mm	10 mm
25 µL (250 µg)	13 mm	10 mm	11 mm
30 µL (300 µg)	14 mm	12 mm	12 mm
Positive control 30 µL (90 µg) (Streptomycin 3mg/ml	17 mm	13 mm	13 mm



Pseudomonas aeruginosa *Klebsiella pneumoniae* *Enterobacter freundii*

Figure 4: Gram - negative bacteria in the Ethyl acetate analysis of Root - *C. ramosissima*

4. SUMMARY AND CONCLUSION

The findings of this study may provide important insights into the medicinal value of *Crotalaria ramosissima* and support its potential application in the development of novel antimicrobial formulations. Identification of effective plant-based antibacterial agents could help address the growing challenge of antibiotic resistance and promote the use of natural

therapeutics in healthcare. Moreover, the study may encourage further phytochemical characterization, isolation of active constituents, and pharmacological investigations of this plant for future drug discovery programs.

Overall, the present research focuses on assessing the antibacterial activity of ethyl acetate extracts from the stem and root of *Crotalaria ramosissima* against three Gram-positive and three Gram-negative bacterial pathogens. The study aims to evaluate the susceptibility patterns of these microorganisms and determine the therapeutic potential of the plant extracts as natural antibacterial agents. The results obtained from this investigation may contribute to the growing field of medicinal plant research and provide a scientific basis for the utilization of *Crotalaria ramosissima* in pharmaceutical and antimicrobial applications.

REFERENCES

Chouhan H S, Singh S K, (2010). Antibacterial activity of seed and flower parts of *Crotalaria juncea* Linn., *American-Eurasian Journal of Scientific Research*, 5(3), 212-215.

Dheeraj, Abhitinder Kumar, Anjali, & Anju (2024). Unveiling the antibacterial properties of plant extracts: Insights and innovations for future therapies. International Journal for Multidisciplinary Research, 6(4), Article IJFMR240426034.

Eloff JN, (1999). The antibacterial activity of 27 southern African members of the Combretaceae, *South African Journal of Science,* 95: 148–152.

Hala Sh. Mohammed, Salwa A. Abu El Wafa, Mona H. Ibrahim, Rasha Mohammad Fathy and Noha A. Seif-Eldein, (2024). *Crotalaria madurensis* flavanol glycosides' antibacterial activity against *Staphylococcus aureus,* 14(118), 1-10.

Hemendra S. Chouhan, Alekh N. Sahu and Sushil K. Singh, (2011). Fatty acid composition, antioxidant, anti-inflammatory and antibacterial activities of seed oil from *Crotalaria juncea* Linn., *Journal of Medicinal Plants Research*, 5(6), 984-991.

Kiruthiga R, Rakkimuthu R and Aravinthan K M, (2014). Antibacterial activity of Crotalaria pallida Aiton. (Fabaceae), Indian Journal of Pharmaceutical and Biological Research, 2(1), 82-85.

Kwaji A, Atiko R and Edeh Friday, (2013). Phytochemical screening and antibacterial activity of crude ethanol root extract of Crotalaria pallida (Aiton) plant, Topclass Journal of Herbal Medicine, 2(10) 235-238.

Mathews Anza Alemu, Habtamu Gelaw Mekonnen and Milkyas Endale Annisa, (2015). Phytochemical analysis and antibacterial activity on seed and pod extracts of Crotalaria incana, Journal of Pharmacy & Pharmacognosy Research, 3 (4),100-108.

Raheela Naseem Raheela Naseem, Karamat Mahmud Karamat Mahmud, Muhammad Arshad Muhammad Arshad, (2006). Chemical composition and antibacterial activity of *Crotalaria burhia*, from Cholistan Desert, Pakistan. Hamdard Medicus, 49(4), 49-52.

Siva Sankar Sana, Divya Vishambhar Kumbhakar, Akbar Pasha, Smita C. Pawar, Andrews Nirmala Grace, Raghvendra Pratap Singh, Van-Huy Nguyen, Quyet Van Le and Wanxi Peng, (2020). *Crotalaria verrucosa* Leaf Extract Mediated Synthesis of Zinc Oxide Nanoparticles: Assessment of Antimicrobial and Anticancer Activity, *Molecules*, *25*(21), 4896; https://doi.org/10.3390/molecules25214896.

Sushobhan Ukil, Subrata Laskar and Raj Narayan Roy, (2015). Physicochemical characterization and antibacterial activity of the leaf oil of *Crotalaria pallida* Aiton, Journal of Taibah University for Science, 10(4), 490-496. https://doi.org/10.1016/j.jtusci.2015.07.001.