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Research Article

# Evaluating *Croton Macrostachyus*: Honey, Nectar, and Pollen Antimicrobial Activities against *Escherichia coli, Shigella boydii, Staphylococcus aureus,* and *Bacillus subtilis*

**Kasim Roba[1*], Zufan Bedewi[2]**

*[1]Department of Honey Bee Forage and Pollination Ecology, Holeta Bee Research Center, Ethiopia Pobox-22, Holeta, Ethiopia;[2]Department of Biology, College of Natural and Computational Science, Hawassa University, Hawassa, Ethiopia*

## ABSTRACT

**Background:** Ethiopia is one of the plant species-rich countries in the world and the centre of origin of many medicinal plants. Studying antimicrobial activities is vital to investigate plants resources for medicinal values and the study was conducted to identify and evaluate *Croton macrostachyus* honey's antimicrobial sources from its nectar and pollen against mentioned bacteria.

**Methods:** Completely Randomised Design was used for the laboratory. After adjusting turbidity, consistent growth of bacterial culture was made using sterilized cotton. The extract of C. macrostachyus' pollen 3.6 gm. was added to 12 ml of distilled water to prepare stock solutions as ppm stock solution and antimicrobial activities of pollen, nectar, and honey were tested against mentioned bacteria above. Data were inserted into Microsoft excel 2010 and imported to R software version 3.44. Multilevel analysis was used to see the interaction between bacteria species and each concentration of honey, nectar, and pollen of Croton and Anova was used to see the significance of these concentrations on bacteria species. A p-value of <0.05 was considered statistically significant.

**Results:** Results indicated that bacteria were more inhibited at 72 hours than 48 and 24 hours and the result identified honey's antimicrobial source was due to pollen composition found in honey that only pollen had antimicrobial activities while nectar had no antimicrobial activities against both Gram-negative and Gram-positive bacteria mentioned above. Time has significant effects on tested bacteria (p=0.000) and treatments have significant effects on tested organisms (p=0.000). Honey inhibited the growth of more Gram-negative bacteria: *Escherichia coli* and *Shigella boydii*. Bacillus was mostly inhibited by crotons' pollen than others.

**Conclusion:** Water extract of Crotons 'pollen had antibacterial activities against all tested bacterial strains, but inhibited more Gram-positive bacteria; while Crotons'honey inhibited more Gram-negative bacteria than Gram-positive bacteria. Negative controls (sterilized water) and nectar did not show an inhibitory effect on tested bacteria, while positive control (Chloramphenicol) had antimicrobial activities. Further isolation and characterization of bioactive compounds from *Croton macrostachyus* pollen are useful to develop a novel botanical formulation for further applications.

**Keywords:** Antimicrobial sources; Crotons honey; Crotons nectar; Croton's pollen; Evaluation; Identification

## INTRODUCTION

C. macrostachyus is a species of the genus Croton. Euphorbiaceae family, commonly known as the spurge family and croton is regarded as a multipurpose tree playing an important role in primary healthcare [1]. Ethnobotanical studies on traditional medicinal plants are the means to increase the capacity of the pharmaceutical industries [2]. Alternative medicine is considered as a primary health care modality in resource-constrained health care settings [3]. Healing with medicinal plants is as old as mankind itself [4]. Pollens are a rich source of essential amino acids and are often considered as a complete food for human beings healthy [5]. According to my knowledge in this field, no data related to the biological activity of Crotons' pollen grain, nectar, and honey has been in literature, and to fill this gap the researcher is interested to do this research. Each flower species has a unique pollen grain [6].

Pollen-based microcapsules such as hollow sporopollenin exine capsules have emerged as excellent drug delivery and microencapsulation vehicles [7]. Comparative study on the antibacterial activities of (pollen, and honey) has antibacterial efficacy against pathogenic Gram-positive and Gram-negative

**Correspondence to:** Kasim Roba, Department of Honey Bee Forage and Pollination Ecology, Holeta Bee Research Center, Ethiopia POBox-22, Holeta, Ethiopia, Tel: +251927983536; E-mail: kasimroba7@gmail.com

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bacteria [8]. Honey contains appropriate antioxidants which are responsible for biological activity, defense, and increase red blood cells functions and pharmaceutical and biological scientists need to identify the floral species which give anti-microbial characteristics [9]. The use of honey as a traditional remedy for microbial infections dates back to ancient times [10]. The reason to study the antibacterial property of honey and pollen is to find safe and natural antibiotics; since several microorganisms have developed resistance to commonly prescribed antibiotics, there is a need to find alternatives [11].

Intoxications and infections caused by food-borne pathogens like *Escherichia coli* represent increasing public health problems; *S. aureus* is a Gram-positive, non-spore-forming spherical bacterium that belongs to the Staphylococcus genus that subdivided into 32 species and subspecies [12]. Shigella species are members of the family Enterobacteriaceae, classified based on biochemical and serological characteristics [13]. The Bacillus species constitute an interesting group of probiotic bacteria that received limited attention [14].

## METHODOLOGY

Selected trees were caged by insects proved to avoid visiting insects to get more pollen for the experiment [15]. A pollen grain of *C.macrostachyus* was collected when it was warm, windy, and particularly when humidity was low and preferably during the middle of the day [16]. Four species of bacteria were selected for the experiment; two Gram-positive: *Staphylococcus aureus* and *Bacillus subtilis* and two-Gram negative: *Escherichia coli* and *Shigella boydii* and they were cultured. With the help of a sterile wire loop, the test bacteria were transferred into test tubes having a sterile nutrient broth and incubated at 37°C for 24 hours until the noticeable turbidity and density was equal to that of 0.5 McFarland standards by adding 0.5 ml of BaCl2 solution into 99.5 ml of H2SO4. After adjusting turbidity, a sterile cotton swab was dipped into suspension and streaked over the whole surface of the plate to make sure the consistent growth of bacterial culture [17].

For the extraction 10, gm. of collected pollen of croton was added to 250 ml of beakers that contains 100 ml of distilled water and shaken for 24 hours and filtered by whatman filter paper and then dried in the water bath. The extracts of Crotons' pollen were measured by sensitive balance and then stock solutions and working concentrations were prepared for its honey and pollen. It was pure honey that was used for the experiment and no need of extracting honey and only working concentrations were prepared by three different concentrations based on the amount of the extract obtained from extractions of pollen. As well, nectar was used directly in all concentrations since no need for extraction nectar. To prepare a stock solution, 3.6 gm. of the extract of C. macrostachyus' pollen was added to 12 ml of distilled water. It was calculated as ppm stock solution. Working concentrations of 100%, 70%, and 30% were prepared from stock.

Antimicrobial activities of C. macrostachyus' nectar, pollen, and honey were tested against mentioned bacteria after they incubated. Disks were mixed into working concentrations of 100%, 70%, and 30% and then added to labeled bacteria species inoculated into Petri dish respectively and stayed for 10-15 minutes until introduced with media, inverted and then brought to incubator incubated at 37℃. The inhibition zone of bacteria was checked continuously for 24, 48, and 72 hours and measured at 48 and 72 hrs. The number of experiments was replicated thrice for all bacteria species and the results were inserted to computer Microsoft excel 2010 and imported to R software version 3.44. Multilevel analysis was used to see the interaction between bacteria species and each concentration of treatments and Anova was used to see the significance of these concentrations on bacteria species. A p-value of <0.05 was considered statistically significant.

## RESULTS

Positive control Chloramphenicol had the highest antimicrobial activities against; *Bacillus subtilis* and *Staphylococcus aureus* than *Escherichia coli* and *Shigella boydii* (Figure 1). Chloramphenicol shows more significant effects than Croton pollen 30% concentrations against mentioned bacteria.

*Bacillus subtilis* and *Staphylococcus aureus* were more inhibited by Chloramphenicol than Gram-negative bacteria: *Escherichia coli* and *Shigella boydii* (Figure 2). Chloramphenicol inhibited more Gram-positive bacteria than Gram-negative bacteria. Chloramphenicol shows more significant effects than Croton pollen 70% concentrations against Gram-positive bacteria but Croton pollen 70% concentrations have more significant effects than standard drug against Gram-negative bacteria.

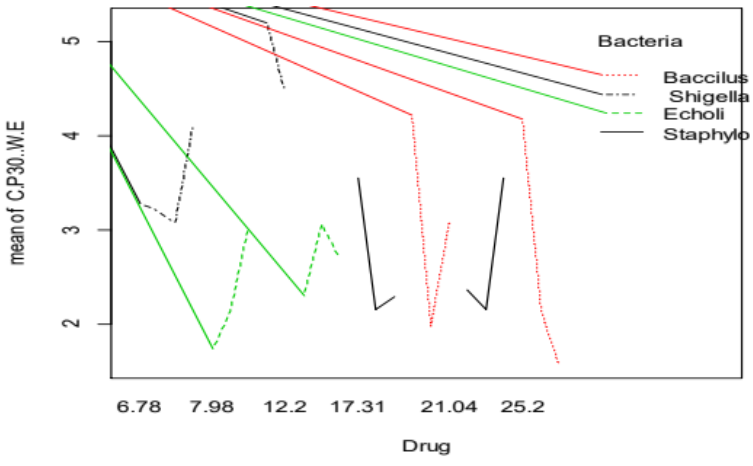


**Figure 1:** Interaction of Chloramphenicol, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and Croton pollen 30% concentrations of water extract.

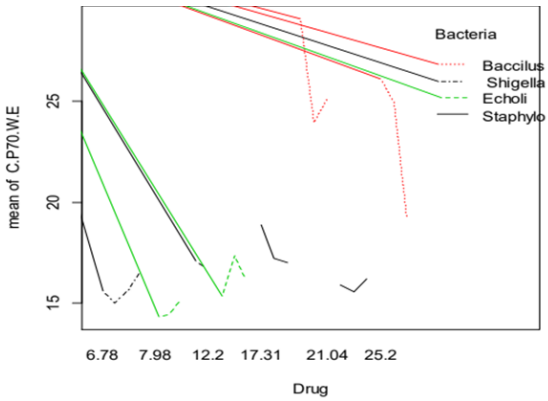


**Figure 2:** Interaction of Chloramphenicol, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and Croton pollen 70% concentrations of water extract.

*Bacillus subtilis* and *Staphylococcus aureus* were more inhibited by Chloramphenicol than Gram-negative bacteria: *Escherichia coli* and *Shigella boydii* (Figure 3). Chloramphenicol shows more significant effects than Croton pollen 100% concentrations against Gram-positive bacteria but Croton pollen 100% concentrations inhibited more Gram-negative bacteria than standard drug.

A 30% concentration of Crotons' honey shows more significant effects than Crotons' pollen 30% water extracts (Figure 4). 30% concentrations of honey inhibited Gram-negative bacteria: *Escherichia coli* and *Shigella boydii* more than Gram-positive Bacillus: subtilis and *Staphylococcus aureus*.

70% concentrations of honey show more significant effects than Croton pollen 70% water extracts against Gram-negative bacteria (Figure 5). Honey 70% concentrations inhibited more Gram-negative bacteria (*Escherichia coli* and *Shigella boydii*) more than Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), but Crotons' pollen inhibited more Gram-positive bacteria (*Bacillus subtilis*).

*Escherichia coli* were more inhibited by honey 100% concentrations than Croton's pollen 100% water extracts (Figure 6). Crotons' pollen 100% water extract inhibited more Gram-positive bacteria (*Bacillus subtilis*) than all the others. Croton's pollen shows more significant effects than honey against mentioned Gram-positive bacteria. Honey 100% concentrations of water extract inhibited more Gram-negative bacteria (*Escherichia coli*) than Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*).

Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were inhibited more than Gram-negative (*Escherichia coli* and *Shigella boydii*) by crotons pollen water extract (Figure 7). Gram-positive bacteria were more inhibited than Gram-negative bacteria by 100% concentration of croton pollen and highly inhibited at t2 than t1.

*Bacillus subtilis* and *Staphylococcus aureus* bacteria were inhibited more than Gram-negative (*Escherichia coli* and *Shigella boydii*) by crotons pollen water extract (Figure 8). Nectar did not inhibit either Gram-negative bacteria or Gram-positive bacteria as shown by the figure below.

*Bacillus subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) were inhibited by honey and it has highly significant effects on all species of bacteria on both Gram-negative and Gram-positive mentioned bacteria (Figure 9). Nectar inhibited neither Gram-negative nor Gram-positive bacteria.

Time of the experiment has highly significant effects on all species of bacteria on both Gram-negative and Gram-positive bacteria (Figure 10). *Escherichia coli* and *Shigella boydii* were mostly inhibited than Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) by honey 100% concentration as shown in the figure below.

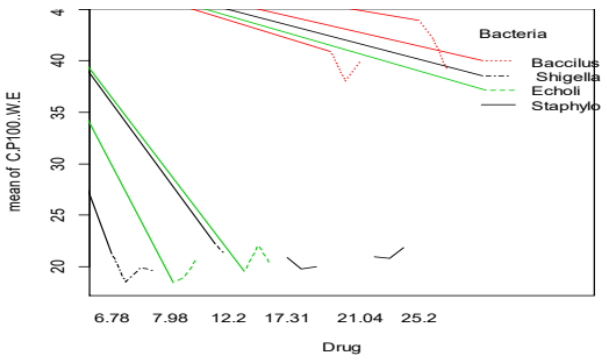


**Figure 3:** Interaction of Chloramphenicol, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and Croton pollen 100% concentrations of water extract.

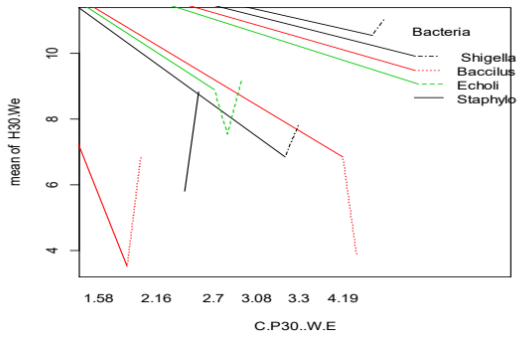


**Figure 4:** Interaction of Croton pollen 30%, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and honey 30% concentrations.

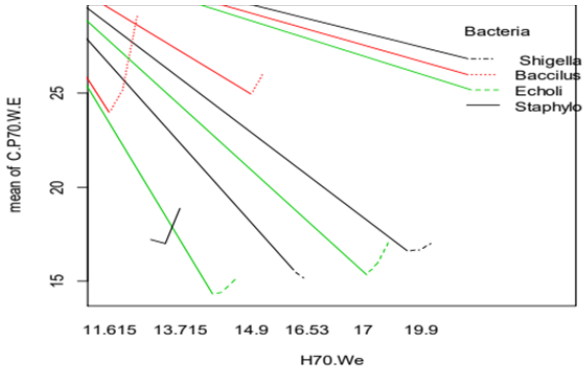


**Figure 5:** Interaction of honey 70% concentration, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and Croton pollen 70% concentrations of water extract.

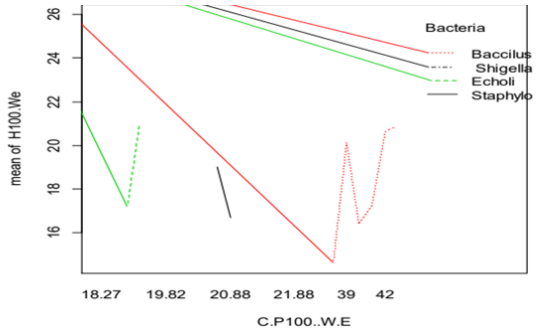


**Figure 6:** Interaction of Croton pollen 100% concentrations, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and honey 100% concentration.

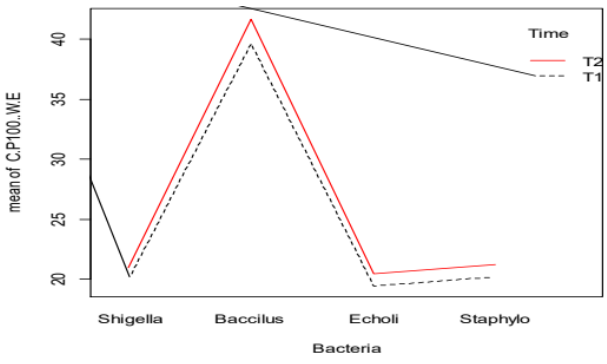


**Figure 7:** Interaction of, *Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*, (T1=48 hours and T2=72 and Croton pollen 100% concentration of water extracts.

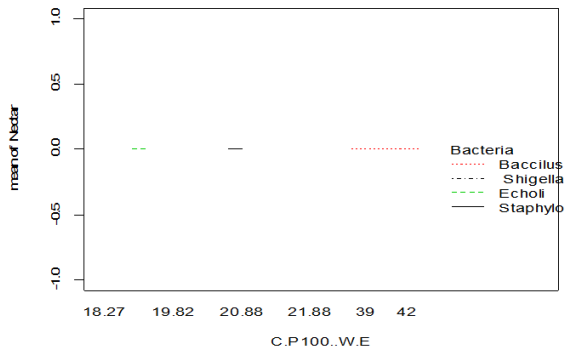


**Figure 8:** Interaction of Croton pollen 100% concentration of water extracts, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and nectar.

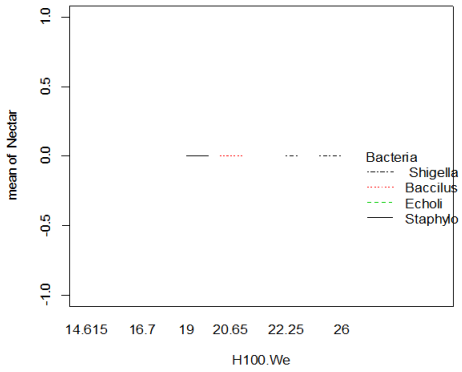


**Figure 9:** Interaction of honey 100% concentration, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) and nectar.

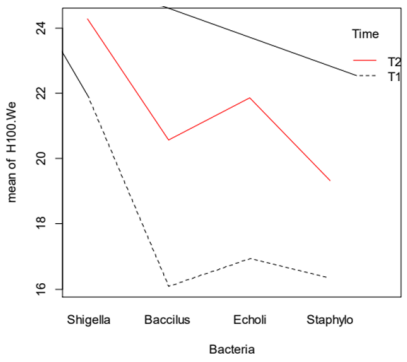


**Figure 10:** Interaction of, (*Bacillus Subtilis*, *Staphylococcus aureus*, *Shigella boydii* and *Escherichia coli*) time and nectar.

## DISCUSSION

The present study summarizes that Crotons macrostachyus' pollen has high antimicrobial effects against both Gram-negative and Gram-positive bacteria however; highly inhibited Gram-positive bacteria (*Bacillus Subtilis*, *Staphylococcus aureus*) than *Shigella boydii* and *Escherichia coli*) at 30, 70, and 100 working concentrations. Honey inhibited more Gram-negative bacteria than Gram-positive bacteria and his result is in line with the reports of [18]. Gram-negative bacteria were more inhibited by 100% concentrations of honey than Gram-positive bacteria. This result is contrasted with the reports of [19]. Pollen 100% concentrations inhibited more Gram-positive bacteria at 72 hours. The antimicrobial activity of the extract is dependent on concentration and exposure periods. Nectar did not inhibit either Gram-negative bacteria or Gram-positive bacteria that might be due to its composition nature that it is carbohydrate bees used as energy sources [20-29]. This study confirmed that honey' medicinal value is due to pollen composition found in it that this study tested antimicrobial effects of honey, nectar, and pollen of croton; honey and pollen has antimicrobial effects while nectar did not show any antimicrobial effects and the study confirmed that it is because of pollen that honey enable to have medicinal uses for many diseases. This study was limited to study antimicrobial activities of croton against these mentioned bacteria and studying many indigenous plants' antimicrobial activities at against more test organisms by different concentration is important to find alternative solutions for resistant developing

bacteria against prescribed drugs [30-38].

## CONCLUSION

In this research program antimicrobial activities of crotons pollen, honey and nectar were tested against mentioned test organisms at concentrations of 30, 70, 100 ppm. Crotons' pollen extract showed antibacterial activities against all tested bacterial strains at the concentrations of 30%, 70%, and 100% and the highest inhibition zone observed on 100% concentration, but in the case of the 48 hours and 70% concentrations of C. pollen solution, a lower inhibition zone is observed at the time of 72 hours than 48 hours against *B. subtilis* that might be due resistance of bacteria. Our study confirmed that this Gram-negative and Gram-positive bacterium inhibition zone differs based on treatment type, working concentration prepared from a stock solution of the experiment, and exposure period of the experiment. We conclude that crotons pollen inhibited more Gram-positive bacteria: *B. subtilis* and *S. aureus* than *E. coli* and *S. boydii*. Honey inhibited more Gram-negative bacteria than Gram-positive bacteria. Honey' antimicrobial sources are from its pollen that no inhibitions were seen by the nectar that might be due to its chemical compositions since it contains carbohydrates which help bees as an energy source; while pollen inhibited both Gram-negative and Gram-positive bacteria. Pollens and honey might be an alternative natural food resource due to their preventative properties. This study is one of the first where an antimicrobial property of Crotons' pollen, nectar, and honey against Gram-negative and Gram-positive bacteria species is quantified. A future study is necessary to pinpoint the functional components in a wide variety of local honey and honeybee forages and test their biological activities to find alternatives for diseases developing resistance against prescribed drugs these days that may be important solutions.

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## ETHICS APPROVAL AND CONSENT PARTICIPATE

The purpose of the study was explained to Dr. Zufan Bedewi the head of the department of natural and computational science at Hawassa University, Ethiopia and she agreed to test antimicrobial activities and identification of honey reasons for medicinal value from its pollen and nectar against mentioned Gram-negative and Gram-positive bacteria.

## FUNDING

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## AVAILABILITY OF DATA AND MATERIALS

The author declares that all other data supporting the findings of this study are available within the article and its supplementary information files.

## AUTHORS' CONTRIBUTIONS

Mr. Kasim Roba did all laboratory work and inserted data to Microsoft Excel and imported it to R software and analyzed and interpreted and wrote this paper for publication and did all necessary things. All authors have read and approved the final manuscript.

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